JOURNAL

OF

HELMINTHOLOGY

(Founded by R. T. LEIPER)

Vol. XXVII, 1953

Edited by

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CONTENTS OF VOLUME XXVII

Nos. 1/2

Nos. 3/4

Journal of Helminthology, Vol. XXVII, *Nos.* 1/2, 1953, *pp.* 1—8.

A Study of Host-Parasite Relationships. The Potato-root Eelworm *(Heterodera rostochiensis)* in Black nightshade *(Solanum nigrum)* and Tomato.

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It is known that root diffusates from *Solanum nigrum* are capable of stimulating the emergence of larvae from cysts of the potato root eelworm, *Heterodera rostochiensis* (Russel *et al.*, 1949). Dr. D. W. Fenwick and Dr. M. T. Franklin have informed the writer that they have found the larvae of *H. rostochiensis* in the roots of black nightshade, *Solanum nigrum* but they were unable to find cysts developing. The present paper is the result of a study of the development of *H. rostochiensis* within black nightshade on the one hand and tomato on the other.

Early in September, 1951, young seedlings of black nightshade and of tomato were transplanted to pots of a three in one loam-sand mixture containing an innoculum of *Heterodera rostochiensis* cysts, collected from an infested potato plot at Rothamsted. The seedlings were roughly comparable in size, but those of *S. nigrum* were slightly the smaller. The pots were stood in a shady position out of doors until the end of September and then moved into a greenhouse as a precaution against frost-damage.

A number of plants of each species was lifted on each of four occasions after the date of transplanting; namely twelve plants at one week, twelve at two weeks, twelve at four weeks and four plants at eight weeks, and two reserve plants of *S. nigrum* and of tomato were allowed to remain in the infested soil for nineteen weeks.

After lifting, the roots were washed, fixed in formal acetic alcohol, weighed after removing surplus moisture and finally stained in hot 0.05 per cent. acid fuchsin lactophenol. Clearing and differentiation were carried out in pure phenol in which the roots were afterwards examined. Nematodes to be examined in detail were dissected from the roots, brought in stages to lactophenol and mounted in this medium.

At the end of a week's growth in the infested soil, three of the twelve plants of black nightshade and ten of the twelve tomatoes had been invaded by the eelworm. A total of 10 larvae were found in the black nightshade and 525 in the tomatoes. Weight for weight, the tomato roots contained about twenty-four times the number of eelworms found in *S. nigrum*. (See Table III). No larvae in either host showed any signs of development at this time.

In the roots of black nightshade there was always more or less necrosis associated with the eelworm. This sometimes showed as a brownish patch on the surface of the root where the larvae had evidently entered, and sometimes as a patch deep in the cortex close to the periphery of the stele. Often the root was constricted or broken across the necrosed area and in many cases the break occurred directly across the position of the larva. The larvae appeared to be fairly evenly distributed and there were no definite swellings on the roots as there were in tomato. In the tomato roots at this stage, necrosis appeared to be less serious and was more generally confined to the surface layers. Where invasion had occurred near the root tip, brown tracks could often be seen in the piliferous layer, apparently marking the preliminary excavations of the nematode before it finally made its entry. Most of the larvae were found in groups a short distance behind the root tip and roots thus attacked almost invariably showed the beginning of lumpy swellings around the invaded parts. On the whole, at this stage, few roots were so badly necrosed that they had actually broken.

From a fortnight onward every plant of the two species examined contained larvae. However, in black nightshade they showed no signs of development at two weeks, while in tomato many had begun to thicken and a few were beginning the second moult. Since Hagemeyer (1951) discovered that the first moult of *H. rostochiensis* occurs within the egg, she thought it probable that the life cycle of this nematode resembles that of *H. schachtii*, as reported by Raski (1950). Throughout this study, therefore, the identification of the different larval stages has been based on Raski's descriptions of *H. schachtii*.

The general condition of the roots of both hosts at two weeks appeared almost unchanged, while 266 larvae were counted in the black nightshade plants and 1,759 in the tomatoes. Comparing equal weights of roots, the infestation is about four times as heavy in the tomatoes as in *S. nigrum*.

At four weeks one larva only out of a total of 917 in the roots of *S. nigrum* was found to be developing. This had just started the second moult and appeared normal in size and internal structure. Even more

FIG.1

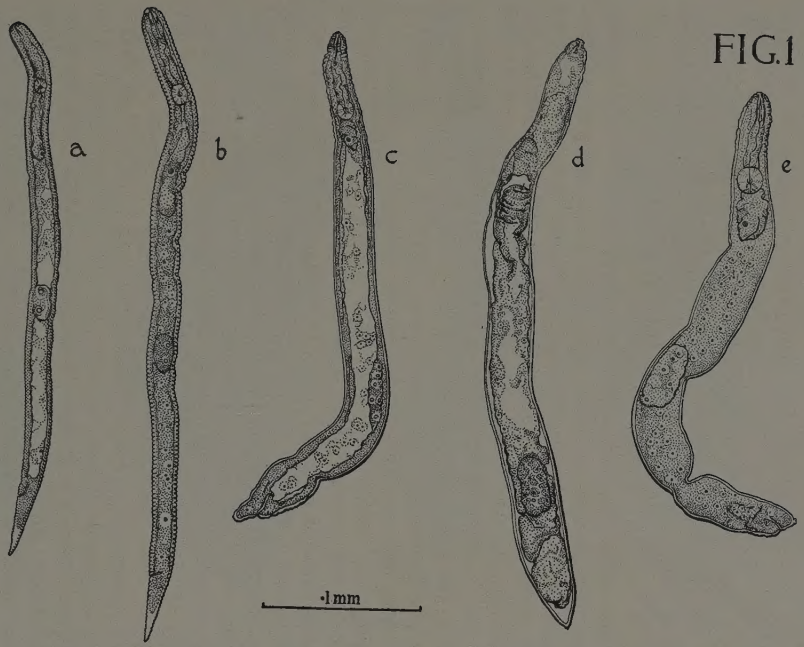

Fig. 1.—a. Degenerating second stage larva from a plant of *Solanum nigrum* grown for eight weeks in infested soil. b. Healthy second stage larva from tomato. c and d. Degenerating third stage larvae from a plant of *Solanum nigrum* grown for eight weeks in infested soil. e. Healthy early third stage larva from tomato.

roots than before were necrosed and broken and almost the only growing points to be seen were on young roots at the distal end of the system and on new laterals just beneath the crown. The tomatoes also showed greater necrosis than before, especially in heavily attacked parts ; many roots were broken off and more patches of deep necrosis were evident. 2,609 *H. rostochiensis* were counted in the tomatoes at this stage, representing just less than twice the number in an equal weight of *S. nigrum* root. The female larvae demonstrated all stages of development up to the advanced third stage (Fig. 2, g) being flask-shaped with elongated ovaries. The males were even more advanced and one which was dissected out was just ready to burst from the fourth larval cuticle as a fully developed adult. (Fig. 2, i.)

At eight weeks, the black nightshade plants were in a more healthy condition than the tomatoes which were stunted and their roots reduced and badly swollen. The nematodes in *S. nigrum* were very scattered and the older roots which had survived attack sometimes showed broad, shallow surface lesions, often with an undeveloped and shrivelled second stage larva in the bottom of them. Out of 2,700 *H. rostochiensis* counted in the four *S. nigrum* plants at this stage, only 12 were found which showed any signs of development. However, some late second stage larvae were almost certainly missed owing to the difficulty of identifying those which lay deep in the roots. Two of the developing forms found were larvae in their second moult, while eight others were third stage larvae which had begun to swell. Three of these showed the first signs of elongation of the genital primordium and were probably males. Structural degeneration was very marked in six of the third stage larvae and the only developing form found in another smaller experiment on *S. nigrum* was an extremely degenerate larva which was probably in the third moult, although the gonad was almost undeveloped. This was found in a plant which had grown for seven weeks in infested soil. All these larvae had little or no food reserves in the intestinal region, which appeared quite void and the structure of the subcuticular layers was, in parts, badly broken down. This degeneration appeared to start in irregular patches in the body-wall musculature between the rectal and oesophageal regions. The patches evidently enlarge and run together in later degenerative stages, leaving only irregular strands of tissue beneath the cuticle. (Fig. 1, c.) These degenerate early third stage larvae were measured and compared with five comparable stages dissected from tomato. The measurements with their means are given in Table I.

FIG. 2

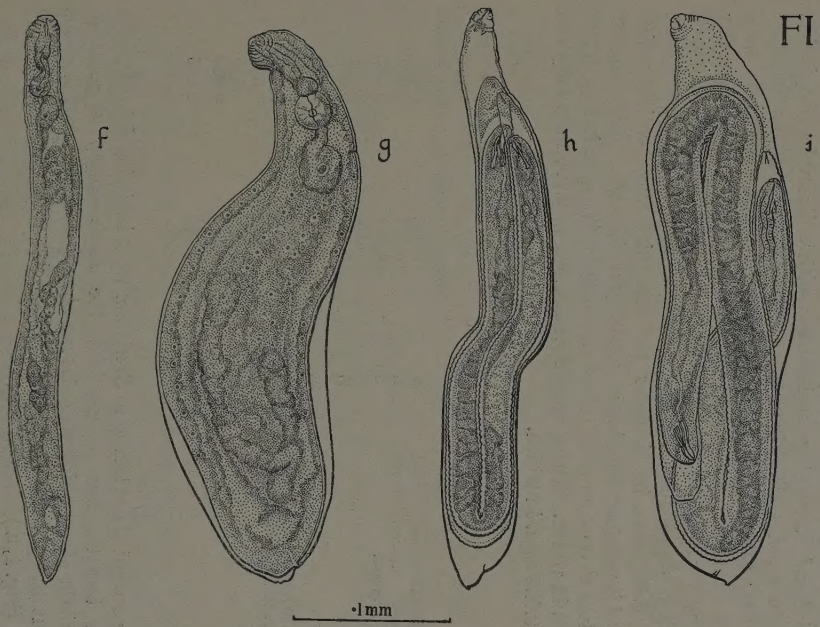

Fig. 2.—f. Degenerating third stage larva from a completely necrosed root of tomato. g. Healthy third stage female larva from a tomato plant grown for four weeks in infested soil. The third moult has just begun. h. Male larva undergoing the final moult. Dissected from a plant of *Solanum nigrum* grown for eight weeks in infested soil. i. Male larva undergoing the final moult. Dissected from a tomato plant grown for four weeks in infested soil.

Probably both of the apparently healthy third stage larvae from black nightshade were males, the more advanced one having a testis 0.197 mm. long but not yet reflexed at the anterior end. One larva had apparently begun the third moult, but it was shrivelled and distorted inside the third larval cuticle and the gonad was little developed. (Fig. 1, d.) The most advanced stage found in *S. nigrum* was an apparently healthy and nearly fully developed male, still enclosed by the third and fourth stage cuticles, but with well-developed spicules, gubernaculum and testis. (Fig. 2, h.) It seems significant that the three most healthy and advanced forms found in black nightshade were males : in a favourable host it is they which normally reach maturity first. However, the fifth stage male from *S. nigrum* was small compared with similar stages dissected from tomato. It measured 0.558 mm. long and 0.021 mm. in diameter and the third stage cuticle which ensheathed it was 0.39 mm. long by 0.48 mm. diameter. Seven similar stages taken at random from tomato averaged 0.872 mm. in length and 0.031 mm. in width. These ranged from 0.985 to 0.766 mm. long and 0.033 to 0.028 mm. in width.

TABLE I.

		Length in mm.	Diameter in mm.
Degenerated early third stage larvae from *S. nigrum*.	1	0.422	0.034
	2	0.361	0.027
	3	0.354	0.032
	4	0.350	0.025
	5	0.346	0.032
	Mean	0.366	0.030
Healthy early third stage larvae from tomato.	1	0.465	0.041
	2	0.425	0.038
	3	0.375	0.032
	4	0.357	0.027
	5	0.354	0.036
	Mean	0.395	0.035

Many second stage larvae in *S. nigrum* were indistinguishable from those in tomato, but others had the same empty appearance characteristic of the third stage larvae already described and it seems likely that these forms represented individuals which were unable to develop. (Fig. 1, a.)

The four tomatoes grown for eight weeks in infested soil had numerous mature cysts protruding from the roots and a total of 1,147 *H. rostochiensis* was counted. The tomato roots were the most stunted and their larval contents still represent a density of infestation nearly

twice that in black nightshade. Only some of the larvae in seriously necrosed parts of the tomatoes showed the same kind of structural degeneration which was apparent in the nematodes from black nightshade. (Fig. 2, f.) However, none was found which was in quite such an advanced state of deterioration.

TABLE II.

The number of *H. rostochiensis* larvae in Black Nightshade and Tomato in relation to weight of roots and to duration of exposure to attack.

	Black Nightshade. Duration of exposure to attack. Weeks.				Tomato. Duration of exposure to attack. Weeks.			
	1	2	4	8	1	2	4	8
No. of plants	12	12	12	4	12	12	12	4
Total weight of roots examined. gms.	0.25	0.43	1.47	3.65	0.55	0.68	2.21	0.85
Total count of *H. rostochiensis*	10	266	917	2,700	525	1,759	2,609	1,147
No. of *H. rostochiensis* per 0.1 gm. root	4.0	61.9	62.4	74.0	95.5	258.6	118.0	134.9

TABLE III.

Relative density of larval population ; Tomato/Black Nightshade per unit weight of root.

Duration of exposure to attack. Weeks.			
1	2	4	8
23.9	4.2	1.9	1.8

The tomato and black nightshade plants remaining in the infested soil for nineteen weeks were all found to be practically free from infestation. The distal roots of the tomatoes were still swollen, but only two or three *H. rostochiensis* were found within each plant. The upper roots were well grown and much thickened and had apparently never been attacked. No trace of any infestation could be found in the plants of *S. nigrum*. This may be partly explained by the fact that the plants had undergone little active growth during November, December and January and that in consequence an active cyst-hatching agent was not being produced (Russel *et al.*, 1949.) Moreover, during November the plants had accidently been allowed to become very dry and

though they recovered, this may have destroyed many of the larvae in the soil which had previously hatched, but not yet invaded the roots. Many of the nematodes in the tomato plants had probably matured and fallen from the roots as cysts, while the larvae which had invaded black nightshade must have died in the necrosed roots, some of which probably broke from the plant. In others new cortex may have been produced beneath the necrosed areas, thus bringing the nematodes to the exterior in shallow surface lesions resembling those found in the eight week plants.

Summary.

Larvae of *Heterodera rostochiensis* have penetrated *S. nigrum*, quickly caused necrosis in the invaded roots and usually themselves suffered degeneration, which it is believed has led to their death. In these cases the region of the intestine apparently becomes devoid of food reserves and the subcuticular layers of the body wall degenerate into irregular strands of tissue. Only of the order of 0.5 per cent. completed the second moult in eight weeks, while the same stage was reached in tomatoes in a little over two weeks. Many larvae apparently do not develop at all. Tomato roots react to invasion by becoming swollen, and necrosis is less in evidence than in black nightshade.

In black nightshade few, if any, larvae reached maturity, but males evidently reached a more advanced stage than females. In tomatoes males reached maturity in four weeks, or just over and females in less than eight weeks. The tomatoes were more readily invaded than the black nightshade plants and the density of the invading eelworm population was about twenty-four times that of black nightshade after one week, about four times after two weeks and twice that of black nightshade at four weeks and at eight weeks.

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Journal of Helminthology, Vol. XXVII, *Nos.* 1/2, 1953, *pp.* 9—16.

Variation in the Nematode Egg Content of Sheep Faeces from Day to Day.

By C. R. W. Spedding, B.Sc.
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The value of faecal egg-counts depends upon the accuracy with which they reflect the size of the nematode burden carried by the host, the degree of the damage being done by the worms, and the rate of deposition of eggs on a pasture. This is subject to a number of influences. Herrick (1928) studying the hookworm of the dog, found that the average number of eggs-per-day (e.p.d.) gave a reasonable index of the number of worms found at autopsy. Egg-laying females are, however, only a part of the worm population, no account being taken of males and immature larval forms not estimated by egg-counts. Kauzal (1937) and Leiper (1951), have pointed out that larvae may exert a considerable influence on the nutrition of the host.

The faecal egg-count, however, provides the only method of estimating the size of worm-burden in a living animal, and to be of maximum value the factors which influence it must be known. The rate of deposition of eggs cannot be defined unless variations in the egg content of the faeces are taken into account.

Spedding (1952) presented the results of an investigation into the variations in faecal egg-content within a single day, as part of a study of the factors affecting the digestive efficiency of sheep. This led to a study of variations which might occur from day to day. In a previous experiment, when faeces were collected from three lambs at 4-hourly intervals for three days, a considerable variation from day to day was indicated. Roberts, O'Sullivan and Riek (1951) stated that " considerable variation in the count may occur from hour to hour and from day to day," and Gordon (1950) concluded that " considerable variations in egg-count occur from day to day even in sheep kept under uniform conditions."

The present experiment was designed to obtain further information as to these daily variations.

Method.

Three Halfbred × Suffolk shearling sheep, previously grazed with the Station flock, were placed in digestibility crates on 21st August, 1951, and fed equal quantities of frozen grass (Raymond, Eyles and Caukwell, 1949). The animals were fitted with leather harness and

close-fitting, rubberised bags, and faeces were collected at 2-hourly intervals for six days. The faeces were weighed, stirred and sub-sampled for egg-counting immediately after each collection. Duplicate counts were carried out on each collection for each sheep. The Zinc-sulphate Centrifuge technique was used as in the previous work, but a horizontal instead of a fixed-angle centrifuge was fitted in this instance. One gram samples were weighed out before any changes in dry matter content of faeces occurred and the samples were placed in glass tubes with 5 ml. of water. The tubes were stored in a refrigerator at 4°C. and slides prepared within 24 hours of collection. In this way the slide to be counted was prepared before any loss of eggs was sustained due to hatching.

Some 450 counts were required, and a method of storing the slides had therefore to be devised to enable counting to take place at a later date. It was considered that this was the correct stage at which to store the samples, the eggs being already isolated on the slide. The method proved entirely satisfactory.

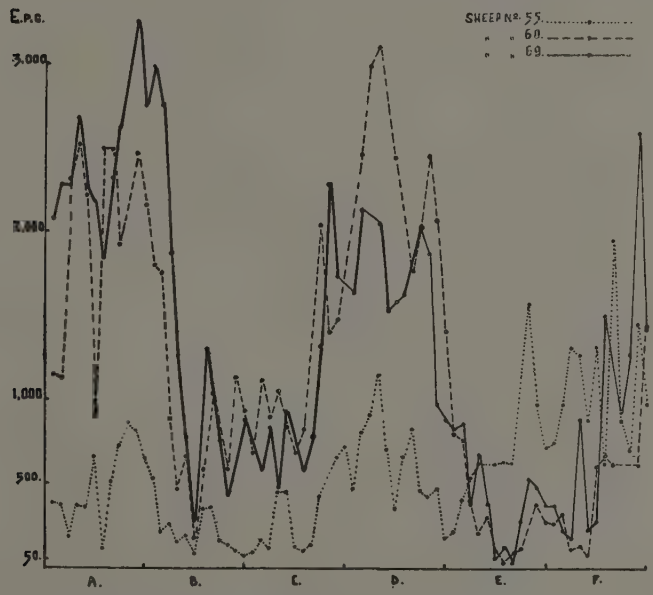
The slides were placed in the refrigerator for 24 hours after preparation. This had the effect of rapidly crystallizing the saturated zinc sulphate at the edges of the cover, thus sealing off the liquid beneath it. When the slides were removed from the refrigerator this temporary seal prevented evaporation and crystallization of the liquid until the crystals at the edges had dried hard and formed a more permanent seal. Longer periods of refrigeration tended to crystallize the whole specimen. In this way a semi-permanent mount was prepared in which the eggs did not hatch or deteriorate sufficiently to render subsequent counting difficult. The deterioration of eggs kept under conditions unfavourable to development, noted by Kauzal (1940), only occurred to a very minor degree. Slides prepared as described have been stored at ordinary temperatures for four months without appreciable change and have overcome the restriction consequent to limited counting facilities and the rather lengthy procedure whereby the eggs in 1 gm. of faeces are counted.

Results.

The e.p.g. mean counts of all three sheep for the whole six-day period are shown in Graph 1. This graph indicates " within day " as well as " day to day " variation.

E.p.g. counts, however, do not accurately reflect day to day changes in egg-output due to the fact that wet weight of faecal output influences the number of eggs in each gram. The eggs-per-day (e.p.d.) data are

therefore shown in Graph 2. The e.p.d. figures were obtained from the number of eggs passed per 2-hourly period (eggs-per-period (e.p.p.) = e.p.g. × faecal weight for that period) by adding the twelve e.p.p. totals comprising the daily output. The total faecal output for each animal is given in Table I, and this shows the effect of faecal weight on



Graph 1.—Mean eggs-per-gram counts of sheep numbered 55, 60 and 69 at two-hourly intervals over a six-day period.

the e.p.d. counts. It will be noted that variations in faecal weight cannot be regarded as responsible for the e.p.d. variations.

All three animals showed very significant variation in both e.p.g. and e.p.p. counts within each day. From day to day the variations were frequently still larger, whether expressed as total e.p.d. figures or as average e.p.g. counts for each day (Graph 3). The latter show the potential fluctuations if the faecal output is not allowed for, even if the correct e.p.g. average is obtained.

The magnitude of the error with egg-counts based on single samples taken on a particular day is shown in Table II. Results are given of

2-hourly counts taken on two successive days, D and E, from Sheep No. 60. The maximum difference observed was between an egg concentration of 3,104 e.p.g. at period 4 (day D) and 45 e.p.g. thirty hours later at period 7 (day E).

TABLE I.

Total daily faecal output (wet wt. in gms.) and eggs-per-day of sheep 55, 60 and 69 over a six-day period, 21st to 26th August, 1951.

Sheep No.		Days A	B	C	D	E	F
55	Faecal wt.	1,873	1,934	1,274	1,570	2,268	1,711
	E.p.d.	989,514	405,350	291,387	908,717	1,461,335	1,985,912
60	Faecal wt.	2,274	2,204	1,704	1,281	2,512	2,335
	E.p.d.	4,666,100	2,117,306	1,970,816	3,111,628	810,314	1,010,614
69	Faecal wt.	1,923	2,627	2,396	2,326	3,018	2,224
	E.p.d.	4,727,222	3,159,252	2,701,010	3,827,726	1,100,832	2,293,373

DISCUSSION.

From the data given it seems evident that the egg-content of sheep faeces cannot be regarded as a constant throughout the day or from day to day. The variations, even over quite short periods are of such magnitude that it is difficult to place any reliance on a single count on one animal. Evidence that a uniform distribution of eggs in the faeces is not to be expected, even if they are laid uniformly, is provided by Gordon (1950). Referring to *Oesophagostomum columbianum* (p. 25) he states: "The worms are often surrounded by mucus into which the eggs may be laid, and masses of mucus are passed with the soft faeces. There are, therefore, regions in which eggs may be aggregated in faeces." This phenomenon is not confined to *Oesophagostomum columbianum*, for he continues: "Females of many of the species in the family *Strongylidae* . . . . have a dark coloured mass of material attached to the vulval region. This mass contains large numbers of eggs, and it appears that the mass is detached from time to time and carries into the faeces an aggregation of eggs which may result in an irregular distribution in faecal samples."

Any increase in the number of samples counted, whether on the same or different animals, automatically increases the significance of the average count. On individual sheep, however, the variations must be taken into account if the egg-count is to be of any use.

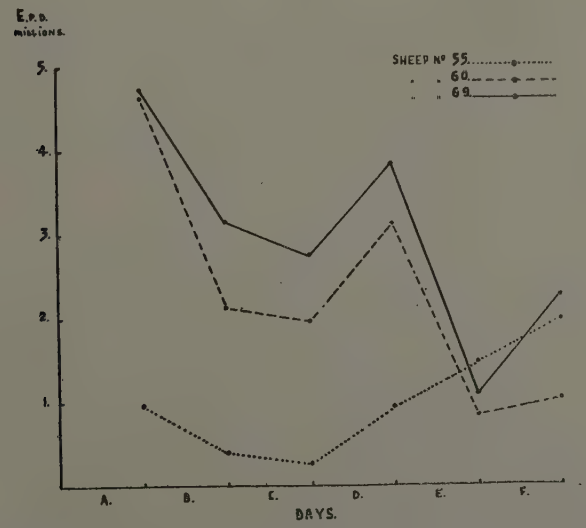
TABLE II.

Faecal output (wet wt. in gms.) and e.p.g. counts of sheep No. 60 at 2-hourly intervals. Days D and E (24th and 25th August, 1951).

Period*		1	2	3	4	5	6	7	8	9	10	11	12
D	Weight of Faeces. (Wet wt. in gms.)	Nil	267		401	Nil	65	Nil	50	62	148	76	209
	E.p.g. ..	—	2,418	2,889	3,132	—	2,650	—	1,581	2,190	2,637	2,277	1,450
	Duplicate ..	—	2,506	3,104	3,076	—	2,258	—	1,963	1,854	2,281	1,871	1,352
	Mean e.p.g.	—	2,462	2,997	3,104	—	2,454	—	1,772	2,022	2,459	2,074	1,401
E	Weight of Faeces. (Wet wt. in gms.)	207	272	207	317	168	135	214	331	73	Nil	145	443
	E.p.g. ..	875	690	365	211	350	128	34	145	137	—	293	229
	Duplicate ..	747	837	452	**	264	73	55	61	114	—	460	304
	Mean e.p.g.	811	764	409	211	307	101	45	103	126	—	377	267

*The periods 1–12 refer to the 2-hourly collection times commencing at 12 noon.

**The duplicate count was lost due to a broken cover-slip during preparation.



Graph 2.—Total daily output of eggs of sheep numbered 55, 60 and 69 over a six-day period.

A comparison of the average e.p.g. figures (Graph 3) with those representing e.p.d. (Graph 2) shows that the former was less reliable. E.p.d. gives a direct measure of the egg-output over the twenty-four hour period. E.p.g. is merely an index of this, distorted by variations in faecal weight and its dry matter content, and subject to other variable factors independent of the faeces.

It is particularly important to note that for any given animal the e.p.g. count parallels the e.p.d. count to a considerable degree, but the parallel is not close enough for comparisons to be made between animals. The levels of infestation in sheep 69 and 60 could not be distinguished with certainty from e.p.g. counts (Graph 3) but were easily distinguishable from e.p.d. data (Graph 2).

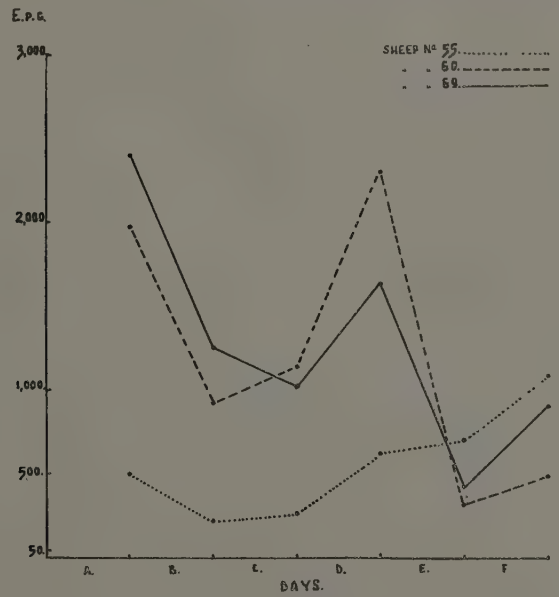
The e.p.d. data also exhibit considerable variations and, if these results are to be of any significance, allowance must be made for these variations. This is possible in two ways. First, counts may be carried out on a number of days and expressed as an average e.p.d. output for the period investigated. Secondly, if the day to day variations follow a rhythm, this may be determined and used to indicate whether any particular e.p.d. count may be regarded as above or below the norm. Tetley (1950) stated that "it was noticeable, over short periods, that appetite, as measured by dry weight of faeces, had a 3 or 4-day rhythm and associated with the rhythm there were fluctuations in the daily output of eggs and also elimination of parasites." It is not clear whether the faecal rhythm may be regarded as responsible for the egg-output rhythm or not, but possibly the egg-laying functions of a worm population conform to a pattern independent of the passage of ingesta or faeces through the alimentary tract.

Evidence of the existence of such a pattern is provided by the present work. It is clear from Graphs 1, 2 and 3 that sheep 69 and 60 exhibited a very similar rhythm in worm-egg production, whether measured in terms of e.p.g. or e.p.d. Sheep 55 conformed to the same pattern for the first four days (A–D) but in e.p.d. the egg-output of day E was too high and did not conform to the results obtained with the other two sheep. Examination of Graph 1 shows that sheep 55 exhibited a region of low counts after the day D peak and that the count rose more rapidly after this than with Sheep 69 and 60.

Employed as a guide to sampling technique this pattern indicates that sampling of all animals should take place at the same time rather than at a particular time. There is nothing in the data to suggest that a given periodicity in egg-output occurs in sheep under different conditions of management. A period of approximately three days separated one

peak of egg-output from the next in the case of the present experiment. Sheep at pasture might exhibit totally different trends.

The solution to such sampling problems is still dependent on the adequate mixing of the total faecal output. At the Grassland Research Station promising results have been obtained with an electrically-driven mixer operating two inter-acting beaters. Using this the total daily faecal output may be mixed, without the addition of a liquid, to give a representative e.p.g. count for the day and, from this, a true e.p.d. figure can be obtained. There seems no apparent reason why an



Graph 3.—Average daily eggs-per-gram counts of sheep numbered 55, 60 and 69 over a six-day period.

aliquot portion of the mixed daily output should not be taken for a number of days and the whole collection mixed a second time. This product may then be sub-sampled and replicate counts carried out to give a true e.p.d. average over the whole period. Such a method could be applied when estimating worm-burden in connection with digestibility trials. It might also eliminate " within day " and " day to day " variations while at the same time considerably reducing the number of counts necessary.

SUMMARY.

1. Variations in the nematode egg-content of sheep faeces are discussed and data from an experiment to study such variations are presented.

2. Previous work on " within day " variations is confirmed and the " day to day " variations are shown to be highly significant.

3. The bearing of these variations on sampling technique is discussed.

ACKNOWLEDGMENTS.

The author's thanks are due to Dr. William Davies for facilities to carry out the work ; to Mr. W. F. Raymond for his advice in the course of the experiment and to Mr. W. I. Jameson and other members of Station staff for assistance in the sampling and recording.

The cooperation of Mr. W. H. Parker (Veterinary Investigation Officer, Wolverhampton) and his staff is also gratefully acknowledged. Duplicate counts were carried out at Wolverhampton and at the Grassland Research Station of the samples taken from sheep 55 for the last three days of the experiment. The two sets of counts were in substantial agreement and provide a valuable check on both the daily and within day variations found.

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Journal of Helminthology, *Vol.* XXVII, *Nos.* 1/2, 1953, *pp.* 17—28.

The Life-History of *Limnaea truncatula* under Laboratory Conditions.

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Knowledge of the life-history of *Limnaea truncatula* is of major interest because of its well-known association with *Fasciola hepatica*, the common liver fluke which causes very great economic loss in cattle and sheep throughout the world. Although the original work of Thomas (1883) on *F. hepatica* and its vector in Britain was followed by numerous investigations both in this country and abroad it was not until Taylor and Mozley (1948) described methods of maintaining the snail in culture at the laboratory that it became possible critically to examine the host-parasite relationship, using snails in numbers adequate for experimental purposes. These methods have proved successful in maintaining a large breeding colony of the snails for a period of four years and it seems of interest to report some of our observations.

Material and Methods.

The many thousands of snails used for experimental purposes at Weybridge all originated from a single mass of eggs laid by a field-snail which was afterwards discarded. In these circumstances a high degree of uniformity throughout the colony may be assumed. The methods of culture, as described by Taylor and Mozley (1948) consist, essentially, of simulating the natural habitat of the snail in unglazed earthenware pans or in shallow glass dishes each of which contains a small pool of water and a mud slope on which grow the green algae which comprise the principal food of the snails.

The observations to be recorded have been compiled from laboratory records of the colony over a period of between three and four years but include also more detailed records of individual snails isolated on mud slopes in culture dishes.

The Life-history of the Snail.

Sexual behaviour: Bailey (1931) suggests that the *Limnaeidae* are the highest forms of animal life, in the evolutionary series, in which hermaphroditism occurs. Furthermore, most, if not all members of the genus *Limnaea* are at least facultatively self-fertile. Even *L. stagnalis* which is so often found in copulation both in the field and in aquaria at the laboratory has been shown (Kendall, 1950) to be self-fertile when kept in isolation. Several observers have indicated that *L. truncatula* may also be self-fertile. Walton (1918) mentioned that the snail was never seen to copulate and we can confirm that, in all our observations at the Laboratory, cross fertilisation was never seen to occur. Individual snails, isolated immediately after hatching, proved fully fertile and, over a period of months, laid large numbers of eggs.

Egg-laying: Where the snails are living under conditions of high humidity, as in the laboratory, the eggs are usually laid on the moist mud of the culture-dish or at the mud-water junction. Under drier atmospheric conditions, such as often occur in the field, the eggs may be found actually submerged in water. In the laboratory, egg-laying has been recorded during every month of the year although it occurs mostly during the warmer months. Seasonal influences are apparent because the food of the snail consists predominantly of algae, the growth of which is conditioned by temperature. It is difficult, therefore, to differentiate between the direct and indirect effects of temperature. Egg-laying has been observed to occur, under experimental conditions at temperatures as low as 10°C. to 11°C. but the culture dishes containing the snails have to be replaced at frequent intervals in order to maintain the supplies of green algae. Most egg-laying undoubtedly occurs at times when plant growth is vigorous and under conditions of good illumination, comparatively high humidity and warmth.

The eggs: Fertility is exceedingly high, the percentage of eggs which fail to hatch being negligible. This is in conformity with Olsen's (1944) observations on the breeding of the related *Stagnicola bulimoides techella*. The number of eggs within each egg mass is variable. Ordinarily each egg contains a single embryo but twinning is sometimes noted. Walton and Jones (1926) gave the presumed maximum number of eggs in egg masses collected from the field as 19. The least number observed was three, the mean of a comparatively small sample being 9.75. Walton (1918) stated that the egg masses contain an average of seven to nine but in our experience the average per egg mass was nearly 14. As will be shown later the number of eggs laid and the numbers

in each egg mass is related to the amount of food available to the snail but under the evidently favourable conditions of our laboratory cultures a single egg mass frequently contained as many as 20 eggs, the largest number recorded being 31. Usually not more than one egg mass was laid each day but two were sometimes noted.

Incubation period of the eggs: This is related to the temperature of the environment, but under average laboratory conditions hatching occurs in about two weeks. At temperatures which ranged between 10°C. and 11°C. incubation occupied 32 days, while at a constant temperature of 11°C. it occupied 29 days. At average laboratory (winter) temperatures of 16°C. to 21°C. young snails emerged in 12–13 days, while during the summer (21°C. to 30°C.) hatching took place in 11 to 12 days.

Growth of the young snails: Soon after hatching the young snails leave the egg mass and commence active feeding. Thereafter, the rate of growth can be extraordinarily rapid. Reference to Figs. 1 and 2 shows that two snails attained shell lengths of 0.48 cms. and 0.43 cms. respectively in 23 days from the date of hatching. This rate of growth was occasionally exceeded as another specimen, under good laboratory conditions, measured 0.5 cms. in a similar period of time. Under these circumstances sexual maturity is reached in about three weeks and the first egg mass is laid. Thereafter the rate of growth decreases (Fig. 1) although the animal continues growing until shortly before death. Fully grown snails at the laboratory commonly attain a shell length of 1.20 cms. to 1.30 cms., a size which is rarely, if ever, exceeded in the field. Roberts (1950) did not observe such large snails in her laboratory cultures and, because she found that field snails measuring 1.00 cms. or more were often infested with *Fasciola hepatica*, suggested the existence of parasitic giganticism. It seems more likely that her snails were maintained in the laboratory under unfavourable conditions and therefore did not attain maximum size.

Aestivation: As shown elsewhere (Kendall, 1949a) *L. truncatula* is capable of surviving for very extended periods in a state of drought-induced dormancy. Aestivation is not, of course, likely to be seen in properly maintained colonies in the laboratory but it is important to remember that it is part of the ordinary life-history of the snail in the field. Consideration of periods of dormancy, whether arising from lack of food or from drought in the environment, is necessary when attempting to assess the longevity of the snail under field conditions.

Longevity, senility and death: There is strong evidence (Kendall, 1949a) that a snail may remain dormant for as long as a year and that

when favourable environmental conditions again obtain it may resume an extended active life, feeding, growing and reproducing. It is apparent that such periods of inactivity must be taken into consideration when attempting to assess the maximum length of life of these molluscs. Under conditions of continuous activity and growth (illustrated by the snail in Fig. 1) the maximum expectation of life appears to be about a year, this being in accordance with the view of Boycott (1936) that "most operculates are presumptive annuals." Some workers, for example Mehl (1932) have given definite limits (10 to 17 months) for the longevity of the snail but it is evident that the period of life is likely to be considerably extended as the result of prolonged periods of dormancy. Any attempt to assess the length of life is therefore useless without a precise knowledge of the environmental conditions and the rate of growth of the particular mollusc. A small snail collected in the field may be either young or old. In either instance it is likely to have a longer expectation of life than a large snail.

An extended period of senility does not appear to be characteristic of the life-history of *L. truncatula*. The snail whose growth is recorded in Fig. 1 remained active until nine days before death and continued egg-laying until 24 days before death occurred.

Factors Influencing the Life-cycle.

Nutrition.

Rate of growth: As might be anticipated the rate of growth (of which the shell length is an index) is quickly affected by the amount of food supplied to the snail. Fig. 1 and Fig. 2 compare the rates of growth of two initially similar snails, both of which increased very rapidly in size for the first three weeks after hatching. Thereafter. one snail (Fig. 1) was moved at frequent intervals to fresh culture dishes, which contained vigorous growths of green algae. The snail received, in addition, a supplementary diet of powdered chalk and oatmeal. Food supplies to the other snail (Fig. 2) were restricted for it was kept in the same culture dish throughout the observation period of nearly a year and it received no supplementary diet. The effect on growth was very marked. The snail receiving adequate food (Fig. 1) grew rapidly to a shell length of about 0.8 cms., and thereafter at a comparatively rapid rate to a maximum length of 1.21 cms. The snail with restricted food grew to 0.8 cms. in a very similar period of time (about 60 days) but since the food supplies in the culture dish were then exhausted, showed very little further growth until its death 361 days

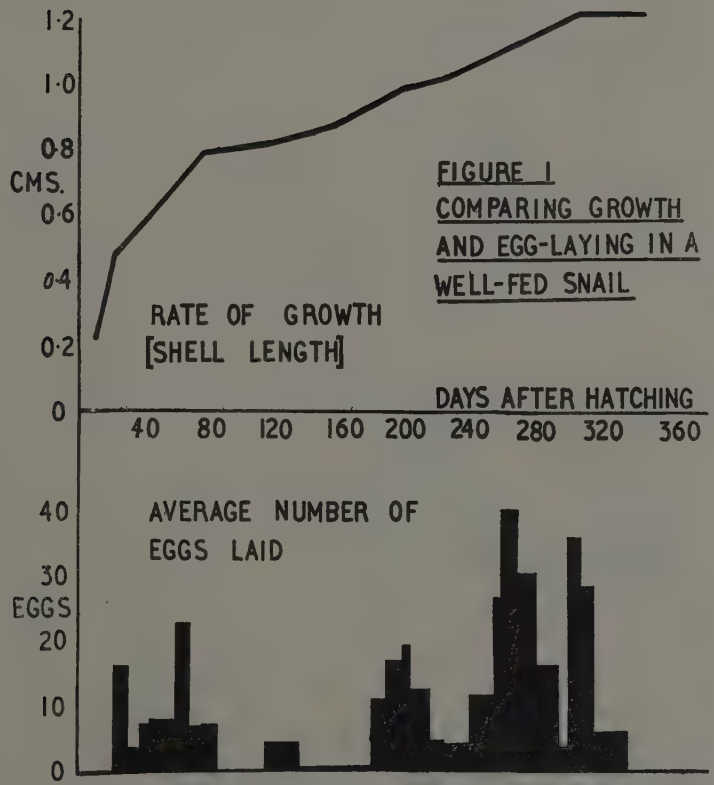

Fig. 1.—Showing the growth and egg production of a specimen of *L. truncatula* that was moved at frequent intervals to culture dishes containing a vigorous growth of algae.

after hatching. Analysis of the growth curve illustrated in Fig. 1 showed that movement of the snail to a fresh culture dish was very quickly followed by an increase in length which could be related to the food supplies of the fresh habitat. Growth within each short period of the life of the snail was so intimately connected with the existing supplies of food that it is evidently impossible to assess the age of a snail by reference to its size without precise knowledge of the ecological background.

Oviposition: It has been shown that shortage of food rapidly affects the growth of *L. truncatula*. Similarly, food supplies influence the number of eggs laid by the snail. Well-fed snails commonly produce an egg mass each day and occasionally two masses, while an aggregate of 50 to 60 eggs may be laid by a single snail during 24 hours. The egg output of the two snails to which reference has already been made (Fig. 1 and Fig. 2) may be compared. It is shown that they produced 3,399 and 894 eggs respectively in about 360 days. Each shaded section of the histiograms (Fig. 1 and Fig. 2) represents the average egg production per day for the particular period of observation. The well-fed snail, as shown in Fig. 1, laid eggs regularly, with the exception of a period in November when green algal growth was scarce in all the culture dishes and the snail suffered from a shortage of food. Apart from this period there appeared to be no seasonal effect on egg-laying. *L. truncatula* under laboratory conditions, like *Fossaria ollula*, whose life-history in the uniform temperatures of Hawaii has been recorded by Alicata (1938), is unaffected by the season. Walton (1918) suggested that the number of ova in each egg mass became less at the end of the period of oviposition. Our observations suggested that larger snails tended to produce more eggs in more egg masses so that, other things being equal, the number of eggs deposited increased with the age and size of the snail. Shortly before death the snails fed less actively so that a reduction in egg production might be expected. In fact the snail recorded in Fig. 1 laid 59 eggs in five masses shortly before dying and the average (twelve eggs) did not differ markedly from that noted at other times during the period of oviposition.

Dormancy: During a period of 29 days (from the 86th day to the 115th day after hatching) the snail whose egg production is recorded in Fig. 1 ceased laying owing to a failure of food supplies. Cessation of egg-laying was associated with a state of dormancy which persisted until the snail was placed in a fresh culture dish containing a large growth of green algae. The snail soon resumed activity and was seen to be feeding on the fourth day and had resumed egg laying on the

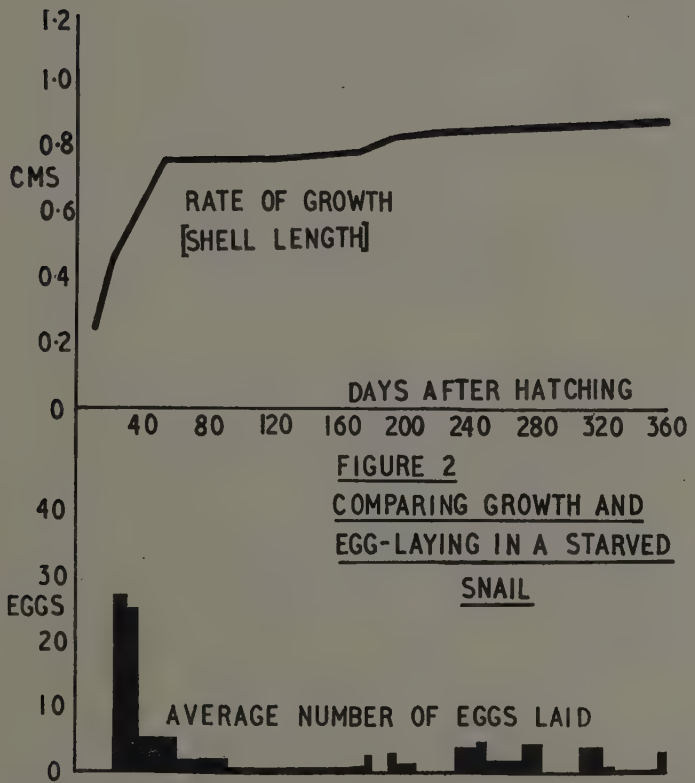

FIGURE 2
COMPARING GROWTH AND EGG-LAYING IN A STARVED SNAIL

Fig. 2.—Showing the growth and egg production of a specimen of *L. truncatula* kept in one and the same culture dish throughout a year.

eighth day after supplies of food again became available. The effect of shortage of food was more clearly illustrated by the behaviour of the snail recorded in Fig. 2. This snail, it will be remembered, did not receive any supplementary food and was kept during its whole life in the same small culture dish. After very active growth during an initial period of about eight weeks, the natural food supplies of the culture dish failed. Thereafter the life of the snail was characterized by periods of dormancy alternating with periods of active feeding on the comparatively restricted algal growth which had developed in the culture dish during successive periods of inactivity of the snail. In preparation for a period of dormancy the snail either crawled up the side of the culture dish (the normal reaction to unfavourable circumstances) or partially buried itself in the mud. In this connection it is interesting to note that mass migrations up the side of a culture dish were noted fairly regularly among the colonies of *L. truncatula* at Weybridge. Young snails were usually involved and it was regarded as an indication of food shortage resulting from over population.

Temperature.

The snail itself is relatively unaffected by low temperatures and remains active at 1.5°C. Low temperatures may, however, have a pronounced effect on the plants supplying food for a colony of snails. For maximum growth and reproduction temperatures ranging between 18°C. and 21°C. appear to be optimum for they assist in maintaining a vigorous growth of the green algae which are essential for successful culture of the snail. Although warmth is necessary for optimum conditions constant temperatures much above 20°C. may be regarded as unfavourable while even occasional temperatures above 25°C. should be avoided. Above 25°C. the snails commonly leave the water of the habitat and remain on the mud with the body extruded from the shell.

The Food of *Limnaea truncatula*.

A precise knowledge of the food of *L. truncatula* would be of the greatest importance if used to define the habitat of the snail. In the field *L. truncatula* usually occurs in interrupted habitats which are sufficiently restricted to make control by the use of molluscicides a practicable possibility. The difficulty is to identify the habitats during periods when the snails are very few in numbers.

At Weybridge it is the practice to supply the colonies of *L. truncatula* with a supplementary diet of oatmeal mixed with finely powdered chalk. Nevertheless the principal food of the snail is the algal growth on the

surface of the mud in the culture dishes. The presence of algae in adequate quantity is undoubtedly the principal factor in the success of the cultures. The species of plant preferred by the snails are still in doubt but on occasion it has been possible to identify particular plants. For example, on several occasions snails were seen to be feeding on the desmid *Cosmarium* in practically pure culture, dissection of the snails showing the plants in various stages of digestion with the hepatico-pancreas containing indigo-blue granules evidently derived from the *Cosmarium*. On most occasions however many and varied forms of plant life, mainly desmids and diatoms, were freely identified on the surface of the mud of the culture dishes and in the crops of the snails. The mere fact that our snails readily ate oatmeal suggests that their natural diet is by no means limited to any restricted class of plant material. Dr. H. B. N. Hynes, who carried out a series of dissections of *L. truncatula* with a view to identifying preferred foods expressed the the opinion (private communication) " I think one can safely state that *L. truncatula* feeds on any plant material it comes across and that it digests it all. Higher plant tissue was probably rasped off dead leaves and the diatoms were certainly being digested."

At the laboratory we are able to distinguish, empirically, between established culture dishes in which *L. truncatula* is likely to thrive and those which are relatively unsuitable. Optimum conditions for the snail appear to occur at an early stage in the ecological succession from bare mud bank to a flora of moss and grasses. Snails thrive at a time when a dense mat of unicellular algae is unobscured by higher plants. Active decomposition of vegetable material or the existence of loosely packed mud in which putrefactive changes are evident are two of several circumstances which lead to the suppression of the early algal growth and failure of the snails to thrive. On the other hand active growth of the larger mosses and grasses or the appearance of dicotyledonous plants leading to the establishment of a more highly organised flora in the culture dish, similarly result in the suppression of the snail. One prerequisite of a successful culture was adequate exposure to light and although we were unable to demonstrate such a spectacular relationship as Raven (1948) showed between the breeding of *Limnaea stagnalis* and its consumption of *Hydrochoris morsus*, it was nevertheless evident that plants in a state of active photosynthesis stimulated the activity of the snails and promoted their growth and reproduction.

Discussion.

Using the methods recommended by Taylor and Mozley (1948) *L. truncatula* did not prove difficult to maintain in culture at the

laboratory. The success of our cultures may be assessed by the very rapid rate of growth and multiplication of the snails and by the low rate of mortality observed among the snail colonies at Weybridge. Perhaps our most significant observations were those indicating the extraordinary prolificacy of the snails. Some previous observers have indicated a relatively low potential rate of reproduction. Roberts (1950), for example, suggested that an average of seven egg masses might be expected from each snail. As already indicated her experimental evidence of growth among laboratory snails suggests that her colonies were maintained under distinctly unfavourable environmental conditions. We have shown that each snail with adequate access to food may reasonably be expected to lay an aggregate of at least 3,000 eggs in about 200 egg masses. This high rate of reproduction allied to the ability of the snail to attain sexual maturity in as little as three weeks from the date of hatching offer an adequate explanation of our laboratory observation that a single snail may give rise to 25,000 in a period of twelve weeks and of the frequently noted and exceedingly rapid recolonisation of field habitats. A high rate of multiplication is essential in order to explain the presence of the snails in numbers as great as those observed by Thomas (1883) who was able to obtain more than 500 with " a single sweep of a small hand net." Such very large populations arise rapidly in places where, but a short time previously, examples were few and far between.

In culture in the laboratory, as in the field, the snail is essentially amphibious, spending much of its time on moist mud banks but entering the water freely, particularly after flooding when it browses on recently submerged algal growth. If snails consistently attempt to leave aquaria in which they are confined (Thomas, 1883) this is a clear indication of the unsuitability of the environment and is usually an indication of food shortage. Walton (1918) showed that snails placed on a sod from a stream margin and supplied with clear water climbed out of the vessel but that if " diatomaceous mud " was added they remained in the vessel and thrived. In our experience snails stay indifferently on land or under water as long as food is present.

The availability of food is the principal factor formulating the habits of the snail and great variations in the food supplies of individual habitats explain many observed discrepancies in behaviour. Boycott (1936) said that food was the factor limiting the number of snails in a particular environment. This is particularly true with *Limnaea truncatula*, all phases of the life-history including the relationship with

the trematode parasite (Kendall, 1949b) being more than ordinarily conditioned by the amount of food which is immediately available.

SUMMARY.

1. This paper describes the life-history of *Limnaea truncatula* under laboratory conditions.

2. Under controlled conditions sexual maturity may be reached in 23 days and egg laying continues for the greater part of the snail's life.

3. Under good environmental conditions each snail may lay as many as 60 eggs in a single day and a total of more than 3,000 in a life of approximately a year.

4. Egg laying occurs during every month and at temperatures as low as 10°C. to 11°C. The incubation period of the egg is related to the atmospheric temperature.

5. The longevity of the snail is likely to be related to its rate of growth and to environmental conditions including periods of drought.

6. The snail remains fully active at temperatures as low at 1.5°C. Sustained temperatures much above 20°C. are unfavourable, temperatures above 25°C. proving markedly deleterious.

ACKNOWLEDGMENT.

This work was directed by Dr. E. L. Taylor who was responsible for many of the original observations recorded.

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Journal of Helminthology, Vol. XXVII, *Nos.* 1/2, 1953, *pp.* 29—40.

The Effect of Repeated Anthelmintic Treatment with Phenothiazine on the Faecal Egg Counts of Housed Horses, with Some Observations on the Life Cycle of *Trichonema* spp. in the Horse.

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Phenothiazine is now generally accepted as the anthelmintic of choice in the treatment of strongylosis in horses and its efficacy has been demonstrated by tests on experimental horses as well as by its extensive use in the field. Only a very small number of critical tests of phenothiazine, involving worm counts, have been carried out in horses and most workers have estimated the efficacy of the drug by a comparison of pre-treatment and post-treatment egg counts; the latter being carried out in most instances less than four weeks after treatment. Knowles and Blount (1940) however, observed that although the post-treatment egg count remained negative for about five weeks, the count then began to rise again and this phenomenon was attributed to the maturation of larvae that were present at the time of dosing and thus out of reach of the drug. Veterinary practitioners in the field have also frequently reported similar experiences, but in many of these instances re-infection might have been responsible for the post-treatment rise of egg count. Since inhibition of egg laying by worms follows the administration of phenothiazine in small daily doses (Gibson, 1945 and 1949), the negative period after treatment might have been explained in this way. It was in an attempt to clarify this position that the investigation reported in this paper was carried out and in order to secure a more certain understanding of the use of phenothiazine for the control of worms in horses.

Experimental Procedure.

Six aged serum-producing horses, which had been kept in loose boxes for six months prior to the beginning of this experiment became available for these observations. The animals were allowed to remain in the loose boxes during the whole experimental period and were fed on hay, bran and oats; grass or other green food likely to be carrying strongyloid larvae being rigorously excluded from the diet. The loose boxes were cleaned out daily, care being taken to remove all faecal

material so that the chances of the animals picking up infection in the boxes were negligible.

A routine of daily faecal egg counts was commenced and continued throughout ; the modified McMaster technique being employed, except when the counts fell below 100 eggs per gramme, when a modified Clayton Lane flotation method was used. A fortnight after egg counting had begun each horse was given a dose of 30 grammes of non-dispersible phenothiazine mixed with a small quantity of damp bran and oats. Following this, as will be explained in detail in a subsequent section of this paper, the egg counts of the horses fell to zero or a very low level but some four weeks afterwards eggs again appeared in the faeces and after a lapse of ten weeks following treatment the count had again reached a high level. At this stage the horses were again treated, horse 97 receiving the treatment in a small quantity of food as before but all the rest received 30 grammes of dispersible phenothiazine in about one pint of water by stomach tube. A similar change of egg count was observed and the phenothiazine treatment was again repeated when the egg count had risen to a steady level. On this and all subsequent occasions, the treatment consisted of 30 grammes of dispersible phenothiazine, suspended in water and adminstered by stomach tube. This sequence of treatment and observation of the changes in egg count was carried out several times during the three year observational period ; horse 96 receiving a total of six treatments, horse 97 six, horse 129 four, horse 130 four, horse 131 five and horse 158 eight treatments.

With the exception of the first treatment, the total faecal output of each horse was collected daily during the week subsequent to dosing, and examined for the presence of worms by the sampling method described in a previous paper (Gibson, 1950). After six counts had been made, however, it became clear that worms were only being eliminated on the second and third days reckoned from the time of dosage ; and on subsequent occasions, therefore, only faeces passed on these two days were collected and examined.

Some three months after the experiment began faeces were collected for purposes of making a differential larval count ; a procedure which continued weekly from that time. Each faecal sample was broken up carefully into a glass culture dish and kept in an incubator at a temperature of 27°C. for seven days. At the end of this time the infective larvae were recovered from faeces using the Baermann apparatus and then a random sample of a hundred was differentiated according to the criteria given by Russell (1948).

At the end of the experimental period horses 97, 129 and 130 were slaughtered for post-mortem examination and the worms present in the large intestine were carefully collected and counted.

RESULTS.

The protocols of this experiment comprise some 5,000 egg counts, 600 differential larval counts and 27 worm counts, and are so voluminous as to preclude their publication in full detail in a paper of this character. The complete figures are, however, being kept at the Veterinary Laboratory of the Ministry of Agriculture at Weybridge where they may be consulted by anyone who may be interested. Some of the salient points are summarised in the following paragraphs together with Figures 1 and 2 and Tables I and II.

TABLE I.

Showing results of worm counts carried out on the faeces of horse 131 for five days after receiving 30 grammes of dispersible phenothiazine by stomach tube on 12.11.45.

Date of collection of faeces	Total weight of faeces passed in ounces	Worms counted in first 8 ounce sample	Worms counted in second 8 ounce sample	Total counted in the two samples	Estimated total of worms in total daily faecal output
13.11.45	361	0	0	0	0
14.11.45	630	35	28	63	2,480
15.11.45	243	6	12	18	270
16.11.45	456	0	0	0	0
17.11.45	504	0	0	0	0

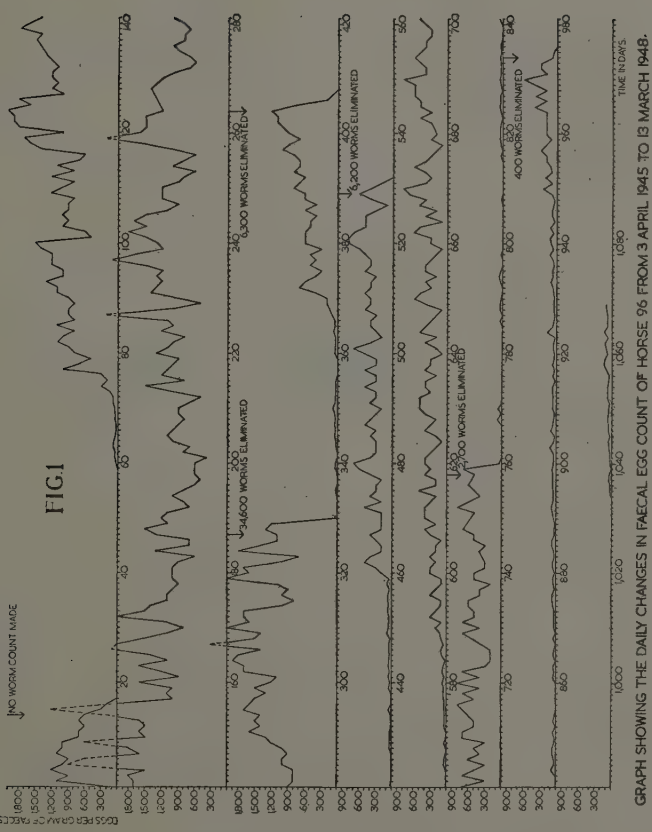
Table I records the results of a worm count carried out on the faeces passed by horse 131 during the five days which followed a 30 gramme dose of phenothiazine given by stomach tube. This count is typical of the series in which such examinations were carried out for the full period of five days after dosing. It will be seen that no worms were passed on the first day or on the fourth or fifth days after dosing, but on some other occasions, however, one or two worms only were passed on the first and again on the fourth days but worms were never found on the fifth day. Ignoring any worms passed on the first and fourth days makes no real difference to the results and accordingly after this finding had been confirmed in six counts, the faeces were collected for counting only on the second and third days after treatment in all subsequent observations.

TABLE II.

A summary of the results of the experiment showing the number of treatments given to each horse, and showing for each treatment the method of administration of the phenothiazine, the number of worms eliminated and the number of days following treatment before the egg count reached 100 eggs per gram.

Horse No.	1st Treatment			2nd Treatment			3rd Treatment			4th Treatment			5th Treatment			6th Treatment			7th Treatment			8th Treatment		
	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram	Method of Administration	Number of worms eliminated	Days after treatment before egg count reached 100 per gram
96	In the food	No count	58	By stomach tube	34,600	37	By stomach tube	6,300	56	By stomach tube	6,200	60	By stomach tube	2,700	191	By stomach tube	400	80+						
97	In the food	No count	42	In the food	6,100	22	By stomach tube	19,800	52	By stomach tube	7,100	39	By stomach tube	2,100	94	—	—	(2) 287+						
129	In the food	No count	64	By stomach tube	5,600	57	By stomach tube	1,200	97	By stomach tube	200	211+												
130	In the food	No count	66	By stomach tube	2,200	113	By stomach tube	(1) 32	158	By stomach tube	300	264+												
131	In the food	No count	67	By stomach tube	2,800	76	By stomach tube	900	126	By stomach tube	900	120	By stomach tube	1,600	248+									
158	In the food	No count	67	By stomach tube	1,800	72	By stomach tube	900	40	By stomach tube	500	66	By stomach tube	(1) 200	67	Drench	(3) 300	3	By stomach tube	600	123	By stomach tube	400	72+

NOTE.—(1) This was an incomplete faecal sample. (2) On this occasion the count fell below 100 eggs per gramme without treatment. (3) The horse only took part of the 30 gramme dose and so probably only a partial effect was obtained on this occasion. (4) All horses received the first treatment on the same day but subsequent treatments were given at varying intervals, each animal being under observation for the same period. During the period of observation, horse 129 received four treatments but during the same period horse 158 received eight.



Note: (1) Phenothiazine treatment was given on days indicated by a black arrow. (2) 30 grams of phenothiazine was given by means of the stomach tube on all occasions except the first when the drug was mixed with the food. (3) By the side of each arrow is recorded the number of worms eliminated as a result of the treatment given on that day.

The results of the egg counts and worm counts have been summarised in Figure 1 and Table II.

Figure 1 gives the results for horse 96, showing the daily fluctuations in egg count, the date of each treatment with phenothiazine and the mode of administration employed, together with the number of worms passed after each treatment.

Table II summarises the results for all six horses. Egg counts are not given in this table but the number of worms eliminated at each treatment and the time which subsequently elapsed before the count rose above 100 eggs per gramme are noted.

Reference to Figure 1 shows that before treatment the faecal egg count of horse 96 was about 1,000 eggs per gramme, but four days after the first treatment with phenothiazine it had fallen to zero. It remained low for 58 days when a rapid rise took place till a count of 1,200 eggs per gramme had been reached at about which level it remained until the second treatment was given. No worm count was carried out on this first occasion so that the number of worms eliminated remains unknown. After the second treatment the count again fell, within three days, to zero and did not rise above 100 eggs per gramme for 37 days after which a rapid rise to 700 eggs per gramme took place. Following this second treatment 34,600 worms were eliminated in the faeces. Four days after the third dose of phenothiazine the egg count was again zero and it did not rise above 100 per gramme for some 56 days, subsequent to which, a further rise took place, the count finally stabilising at about 500 eggs per gramme. This third treatment resulted in the elimination of 6,300 worms in the faeces. The animal was then dosed a fourth time and on this occasion 6,200 worms were eliminated and four days afterwards the count had fallen to zero. It was 60 days before the count rose above 100 eggs per gramme, it then quickly rose to 600 eggs per gramme, at which level it stood when the fifth treatment was given. This time 2,700 worms were eliminated and the count fell to zero three days after treatment. It was 191 days before it rose again above 100 eggs per gramme and by the time the sixth dose of phenothiazine was given it had reached only 300 eggs per gramme. On this occasion 300 worms were eliminated and the count remained less than 100 eggs per gramme for the next 80 days after which the experiment ended.

Reference to Table II shows that similar results were obtained with the other five horses.

Interesting changes were observed in the differential larval counts

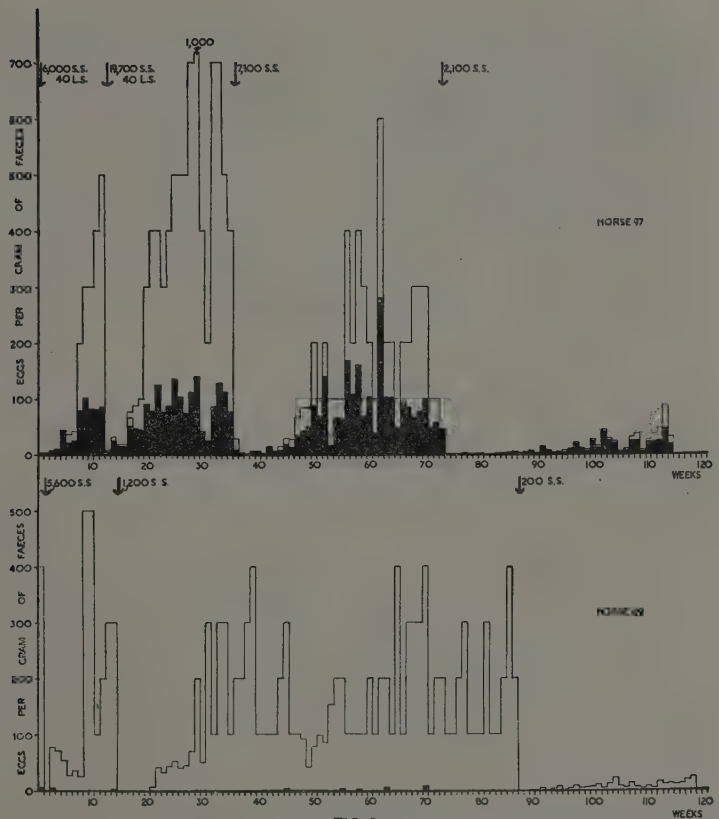


FIG. 2

SHOWING THE WEEKLY CHANGES IN FAECAL EGG COUNT OF HORSES 97 AND 129

Note: (1) The black part of the vertical columns represents the count of eggs of large strongylid worms and the unblackened part represents the count of eggs of small strongyles. The figures were arrived at by dividing the egg count in the same proportion as that shown by a differential larval count carried out on the same sample of faeces. (2) Treatment with phenothiazine is indicated by a black arrow at the appropriate point. By the side of the arrow is recorded the number of worms eliminated by the treatment. The abbreviation S.S. indicates small strongyles and L.S. indicates large strongyles. (3) Differential larval counts were carried out weekly over only part of the total period of observation. Only this shorter period is recorded in this diagram.

carried out on the faecal samples from the horses and these are illustrated in Figure 2. The top diagram of Figure 2 shows the differential larval count for horse 97 and this is generally typical of the whole series, except for horse 129 which was peculiar in carrying an almost pure infestation of *Trichonema* spp. The differential count for horse 129 is represented in the lower diagram of Figure 2. The columns in Figure 2 represent the faecal egg count on the day the differential count was made and each column is divided into a solid black portion at the bottom representing the egg output of the species of *Strongylus* and an unblackened part at the top which represents the egg output of the group of small strongyles, consisting of species of *Trichonema*, *Gyalocephalus*, *Poteriostomun*, *Triodontophorus* and *Oesophagodontus*. Referring to the diagram for horse 97 it is seen that after treatment the egg count remained at zero for a short time and then for several weeks only eggs of *Strongylus* spp. were found in the faeces. After this period eggs of the small strongyles appeared and increased rapidly in numbers until finally about 80 per cent. of the total count represented small strongyles and only 20 per cent. represented *Strongylus* spp. It will be observed that this cycle of events was repeated after each treatment except on the last occasion when the proportion of small strongyles rose to only about 30 per cent. of the total. The lower diagram in Figure 2 shows that *Strongylus* spp. were almost entirely absent from horse 129 and in this instance the egg count remained at zero for five or six weeks following treatment after which it began to rise. The differential count showed that almost all the eggs passed were those of the small strongyles and the diagram is very similar to that for horse 97 if all the eggs passed by *Strongylus* spp. are omitted from the latter.

Table III.

Showing the number of worms found at post-mortem examination in the large intestines of three horses killed at the end of the experiment.

Horse	Large Strongyles	Small Strongyles
97	105	817
129	7	519
130	9	174

Post-mortem examinations were carried out on three of the horses at the end of the experiment and the number of worms found in the large intestine of each is shown in Table III.

DISCUSSION.

It will be observed from a study of the results recorded above that after each succeeding dose of phenothiazine fewer worms were eliminated, a longer period elapsed before the egg count rose above 100 eggs per gramme, and the egg count became stable at a lower level after treatment than before. The first eggs to appear in the faeces after treatment were those of *Strongylus* spp. to be followed later by those of the small strongyles ; the latter increasing until finally they represented about 80 per cent. of the total.

Three explanations have suggested themselves and these are discussed below. The first is that the rise in egg count following treatment was simply due to reinfestation, the larvae being picked up and developing to maturity subsequent to treatment. Since the horses were housed in loose boxes which were thoroughly cleaned each day throughout the experiment and fed on hay, bran and oats the possibilities of reinfestation are small and can be ruled out as a significant cause of the rise of egg count after treatment. As well as the conditions of management of the horses being such as to reduce reinfestation to negligible proportions the results of the experiments provide a potent argument against this suggestion, for it is difficult to believe that the food or the loose boxes would contain progressively fewer larvae throughout the whole course of the experiment so as to result in a lower level of infestation after each treatment.

A second possible explanation is that initially each horse had a heavy worm burden and that only a proportion of this was removed by the first dose of phenothiazine, the egg laying ability of the remainder being depressed by the action of the drug. Subsequently, as these latter worms recovered their power of egg laying, a rise in egg count would occur. As a portion of the worm burden was removed at each treatment so the rise in egg count would be smaller after each treatment. Critical tests (Gibson, 1950) have, however, shown 30 grammes of phenothiazine to be 100 per cent. effective against small strongyles so that the large number of small strongyles eliminated after the second and subsequent treatments could not have been left over from the first one.

The third suggestion depends on the hypothesis that larvae of *Trichonema* spp. may be inhibited in their development and remain for long periods in the mucous membrane of the large intestine. This inhibition of development may be due to the influence of mature worms present in the lumen of the intestine and accordingly, when this influence

ceases to operate after the mature worms have been eliminated by a dose of anthelmintic, the dormant larvae are able to leave the mucous membrane and develop to maturity. When these larvae reach maturity a new influence would operate tending to inhibit further larvae leaving the mucous membrane. This state of affairs would persist until a further dose of anthelmintic removed the new infestation of mature worms and the cycle of events would be repeated many times until the stores of dormant larvae in the mucous membrane became exhausted.

If this hypothesis is accepted the sequence of changes of egg count observed in the six horses used in this experiment can be explained in the following manner : initially the horses were carrying a heavy worm burden, there being many mature worms in the gut lumen and large numbers of larvae in the mucous membrane of the gut wall. As demonstrated by critical tests (Gibson, 1950) the first dose of phenothiazine would remove all the small strongyles from the gut lumen but would be only partially effective against *Strongylus* spp. The egg laying ability of the *Strongylus* spp. left behind would, however, be inhibited by the phenothiazine so resulting in a negative egg count after treatment, but when these worms regained their power of egg laying, eggs would again appear in the faeces and it was observed that the first eggs to appear after treatment were those of *Strongylus* spp. After the removal of the mature small strongyles from the intestinal lumen the dormant larvae begin to leave the mucous membrane and develop to maturity. At this stage eggs of the small strongyles appear in the differential count and the proportion of the count represented by small strongyles increases until larvae cease to leave the mucous membrane. The egg count now becomes stable and this process is repeated each time a dose of phenothiazine is adminstered so resulting in a gradual depletion of the stores of larvae in the mucous membrane until finally, after some six or eight treatments, almost all the larvae will have developed into mature worms. It is clear that after each treatment the egg count will not rise to such a high level as it was before dosing and the number of worms eliminated at each successive treatment will be smaller. After many treatments with phenothiazine it would be expected that few, if any, larvae would remain in the mucous membrane, and in fact, this was the finding in those horses upon which a post-mortem examination was carried out.

Where larval migration into the mucous membrane of the gut wall constitutes a normal part of the life cycle of a nematode—the " histotropic phase " (Kotlan, 1952)—it is usual for the larvae to return to the gut lumen within a certain specified period, generally only a

matter of a few days. Larvae which do not return within this period are usually encapsulated and rendered incapable of further development. The presence of nodules, formed in this way has been described by many workers and Sarles (1944) working with *Oesophagostomum columbianum* and Andrews (1939) working with *Cooperia curticei* suggested that nodule formation and encapsulation of the infective larvae is a manifestation of acquired resistance on the part of the host. Kotlan (1952) in a discussion on the histotropic phase of development in nematodes indicates that a prolonged histotropic phase is irregular and that usually such a condition is accompanied by changes of a chronic inflammatory nature leading to encapsulation of the larvae.

It now appears, however, that pathological changes in the mucous membrane detrimental to the larvae may not always follow when return of the larvae to the gut lumen is delayed. Larval development may be simply inhibited for a variable period of time and continuation of development may take place when suitable conditions obtain. Although the observations recorded in the paper appear to be satisfactorily explained only on this basis further experimental proof is required before the inhibition of larval development in the mucous membrane can be regarded as finally established and it is hoped to carry out this work in the future.

The practical implications of these conclusions are that a single dose of phenothiazine cannot be relied upon to rid horses of their worm burden even though the animals may be kept under conditions where reinfestation is unlikely but several treatments are required to reduce the worm burden to a low level. An egg count performed two or three weeks after treatment will give no indication of the development of a new worm burden as a result of inhibited larvae leaving the mucous membrane and developing to maturity subsequent to treatment, and the necessity for retreatment can only be assessed by an egg count carried out some eight or ten weeks after the previous dose of phenothiazine.

Summary.

It was found that in six aged horses after treatment with 30 grammes of phenothiazine the faecal egg count fell to zero but had returned to a high level after some five or six weeks. Examination of the faeces at the time of treatment showed that many worms were eliminated. Repetition of the treatment several times produced a similar result on each occasion, but there was a tendency for the egg count not to rise so high as it was before treatment, for fewer worms to be eliminated at

each treatment, and for the time before the egg count reached a high level after treatment to lengthen with each successive treatment. Many treatments had to be given over a period of some three years before the egg counts of the horses were reduced permanently to a low level. Post-mortem examination of three of the horses at this stage showed their worm burdens to be low.

In discussion it is suggested that this sequence of events can only satisfactorily be explained by assuming that the development of large numbers of larvae is inhibited in the histotropic phase and that they only leave the mucous membrane of the caecal wall after the adult worms in the gut lumen have been removed by an anthelmintic. Gradual depletion of the stores of dormant larvae over a period, as successive doses of phenothiazaine are given, results finally in a low worm burden in the horse and the almost complete freedom from larvae of the caecal mucous membrane. Where control of worms is being carried out, the necessity for repeated treatment of horses, even when not exposed to reinfestation, is pointed out and the necessity for checking the worm burden about ten weeks after treatment is stressed.

ACKNOWLEDGMENTS.

Thanks are due to Dr. E. L. Taylor and Mrs. A. F. Gush for many useful discussions on the new theory put forward in this paper.

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Journal of Helminthology, Vol. XXVII, *Nos.* 1/2, 1953, *pp.* 41—74

Chlorphenol and Related Compounds as Nematicides I.

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In a recent paper Staniland (1950) showed that iodine and chlorphenol were valuable nematicides but because chlorphenol has a maximum solubility of only 0.5 per cent. difficulties arose when the method was applied commercially. It was thought that emulsions of chlorphenol might be more rapidly soluble in water, when diluted, and various emulsification methods were tried. The best results were obtained with proprietary detergents of the long-chain alkyl-sulphate type. In an attempt to improve an emulsion by the addition of more detergent, it was noticed that the emulsion suddenly cleared and apparently gave a clear solution. As the amount of chlorphenol present was in excess of maximum solubility, an explanation of the phenomenon was sought. Thanks are due to Dr. H. H. Eley of the Department of Physical Chemistry, University of Bristol, for explaining that this was an example of a micellar solution, or " solubilization " (McBain, 1950). In addition to solving the solution difficulties, it seems likely that solubilization opens up many possibilities for improvements in eelworm control. By means of a detergent it is possible to effect solubilization of certain chemicals at strengths in excess of normal solubility and in a form in which the material will readily penetrate membranes. The work has therefore been extended to cover a range of chemicals including chlorphenol (Staniland and Stone, 1952).

The purpose of the present paper is to summarize the progress of the work up to the stage at which a full time chemist was appointed to assist in the investigation ; thanks are due to the Agricultural Research Council for the grant which has made this possible. The appointment is held by Miss J. K. Bartlett who is now working in this department at Bristol. Miss Bartlett commenced work in time to assist with some of the work included in this paper.

The Treatment of Seeds with Solubilized Chlorphenol.

The experiments in the treatment of clover and teazle seed described in a previous paper (Staniland, 1950) were repeated in order to be sure that no ill effects as regards germination took place as the result of the replacement of chlorphenol in solution by solubilized chlorphenol.

The chlorphenol was again used at a strength of 0.5 per cent. and was solubilized with detergent at a strength of 0.25 per cent. A gallon of the solution is made as follows :—

From the bulk of the water about $\frac{1}{2}$ pint is poured into a separate vessel. To this is added 11 ccs. (approximately two-fifths of a fl. oz.) of the detergent. Then 22 ccs. (approximately four-fifths of a fl. oz.) of chlorphenol is stirred in, the result being an emulsion. This emulsion is then poured into the remaining bulk of water and again stirred ; the chlorphenol then passes immediately into micellar solution.

As a guide to the volume of liquid required to treat any given volume of seed, approximately 100 volumes of seed require some 60 volumes of solution in order to cover the seed throughout the soaking period of 20 minutes.

The cycle of operations is again set down :—

(1) The seed is placed in a vessel fitted with a tap in the bottom so that liquid may be drained off when required. The base of the tap on the inside of the vessel is protected with metal gauze to prevent blockage.

(2) The seed is covered with the chlorphenol solution, making sure that all the seed is well wetted.

(3) The seed is allowed to soak for 20 minutes.

(4) The chlorphenol solution is run off.

(5) The seed is well rinsed through with clean water to remove the bulk of chlorphenol.

(6) The seed is allowed to drain for a few minutes and is then removed, mixed with roughly the same volume of dry sand and spread out to dry. In a few hours the seed may be separated from the sand by means of a fine sieve. The seed alone will dry completely in a further short space of time and may be sown as soon as it will run in a drill. As an alternative seed and sand may be sown together, when dry enough to run, and so save time in sieving.

This method is designed for the use of farmers who wish to treat reasonable quantities of seed, which is dried sufficiently for sowing purposes and then sown. The results of these further experiments show that the use of solubilized chlorphenol does not reduce the

germination of the seed, when compared with the use of ordinary water solutions.

The Use of Solubilized Chlorphenol in Bulb Baths.

It has already been stated that solubilization of chlorphenol had solved the difficulties of slow or imperfect solution, so that solution could now be made quickly and efficiently in the cold. The use of chlorphenol in the water of bulb baths overcomes the difficulty of ensuring a complete control of all eelworm, particularly that in the " wool " stage ; the chemical is assisted by the detergent, which ensures that all eelworm " wool " is fully wetted early in the treatment. " Wool"' besides being situated in masses around the edge of the base plates of bulbs, is also to be found within the semi-dry tissues of badly damaged base plates. The detergent creeps into this honeycombed

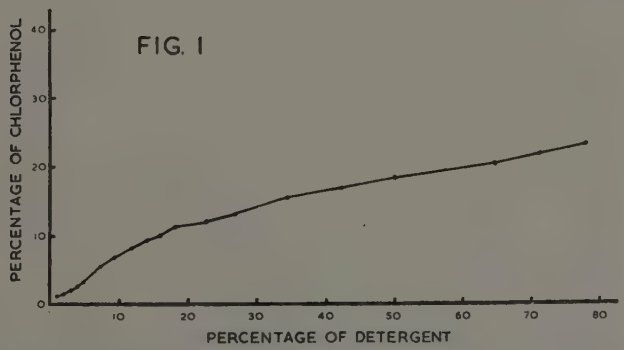

Fig. 1.—Graph showing the relationship between percentage concentration of detergent and percentage " solubilization " of chlorphenol, up to a strength of approximately 20 per cent.

tissue, the chemical in solubilized form being carried with it. When eelworm " wool " was subjected to a temperature of 110°F. for 3 hours, there was no mortality of the worms when the " wool " was kept dry during the process ; in the same way dry " wool " could withstand 2 hours' treatment at a temperature of 115°F. without harm. Air bubbles readily attach themselves to bulbs, particularly to the base plates and these bubbles are probably responsible for imperfect wetting of eelworm " wool." In a three-hour treatment there is always the possibility that " wool " may not be wetted sufficiently early for it to receive a treatment of 78 minutes at that temperature, whilst in a

wetted condition. When no detergent is used, air bubbles continue to rise throughout a treatment; but when a detergent is used, by its wetting action bubbles cease to rise after a short while.

The times for killing wetted eelworm "wool" at a temperature of 110°F. were re-determined over a range of concentrations of solubilized chlorphenol; this was done by methods described elsewhere (Staniland, 1950). The time taken to kill "wool" at 110°F. was sufficiently reduced when the solubilized chlorphenol was used at as low a strength as 0.1 per cent.; at this concentration the time required was 19 minutes, as compared with the time of 28 minutes when an ordinary solution was used at the same strength, and a period of 78 minutes when treated at a temperature of 110°F. in water alone.

The quantities and the method of mixing of the chlorphenol solution is as follows:—

The detergent is used at the minimum rate of 1½ pints per 100 gallons (0.16 per cent.) for the correct solubilization of the chlorphenol at 0.1 per cent. In practice the detergent may be used at 0.25 per cent., thus allowing a small excess for both efficiency in wetting and of solubilization. The quantity of detergent for 100 gallons at a strength of 0.25 per cent. is 2 pints.

From the 100 gallon bulk of water pour about 2 gallons into a separate vessel. To this add 2 pints of detergent and mix well. Then pour in 16 fl. ozs. (eight-tenths pint) of the chlorphenol and mix thoroughly. An emulsion is produced and this is stirred into the bulk of water and then passes at once into micellar solution.

It is important that bulbs should not be treated with the addition of chlorphenol solution unless they are thoroughly dormant. Once root formation has commenced, damage is much accentuated if chlorphenol is used. It is not suggested that all bulbs should necessarily receive a chlorphenol addition when they are hot water treated; such a treatment can be reserved for the more severely attacked bulbs where there is reason to suspect the presence of eelworm "wool."

Care in the Handling of Chlorphenol.

Concentrated chlorphenol can burn the skin severely and rubber gloves should be worn when handling the concentrate. Dilute solutions of 0.5 per cent. or lower do not affect the skin except when the hands are constantly being wetted over a long period as in bulb treating, where, in addition, the solutions are warm; rubber gloves should be worn in these circumstances.

Chlorphenol is harmful to fish and it should not be allowed to run direct into ditches, ponds or rivers. Disposal is best effected by

running used solutions into a hole in the ground, filled with clinker.

Solubilization.

Following the discovery of the solubilization of sparingly soluble benzene derivatives by long chain alkyl sulphates, estimates were made of the quantities of each material required for different concentrations.

When a derivative such as chlorphenol is added to a mixture of detergent and water, a certain amount is held in micellar solution and the mixture remains quite clear and free from droplets. As more

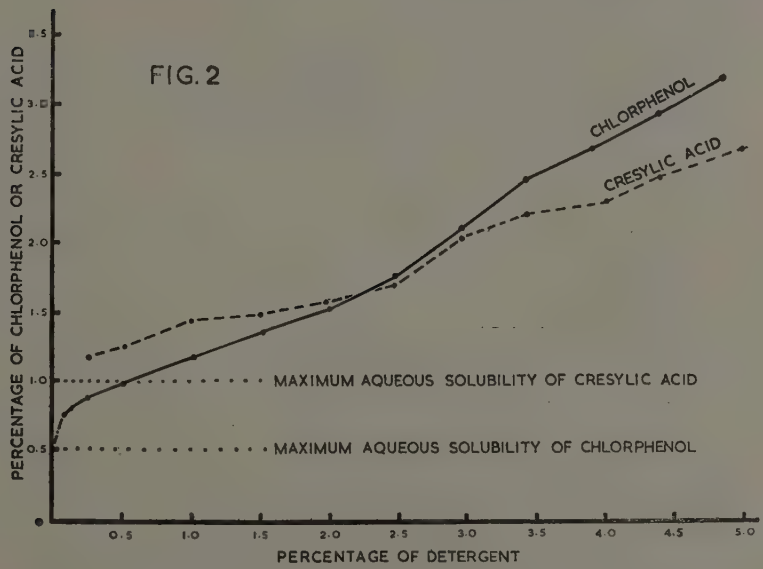

Fig. 2.—Graph illustrating the " solubilization " of chlorphenol and cresylic acid, up to strengths around 3 per cent., by means of a detergent.

derivative is added, a point is reached when no more can be solubilized. An emulsion is then formed and the mixture turns milky. If still more is added, the mixture becomes unstable ; the derivative and detergent come out and form a layer below the water, the latter being a saturated aqueous solution of the derivative.

Using a proprietary alkyl sulphate as detergent, the behaviour of a chlorinated phenol over a wide range of concentrations was investigated in the following manner. A graded series of mixtures of detergent and

water was made up in measuring cylinders. The chlorphenol was then added to each, a little at a time ; in between each addition the mixtures were agitated thoroughly by means of a perforated zinc disc mounted on stiff wire. The volume of chlorphenol added, prior to the first signs of milkiness, was noted in each case. The volumes of chlorphenol and detergent were then converted into percentages of the total volume of liquid in the mixture. Fig. 1 shows the relationship between these percentages and concentration. It will be seen that, as concentration increases, a greater proportion of detergent to chlorphenol is necessary to effect solubilization.

Whilst obtaining this information, an interesting phenomenon was observed. At the higher concentrations, before micellar saturation is reached, the mixture becomes thick and viscous, like clear vaseline. By further additions of chlorphenol, the mixture first thins down again and remains clear, then becomes milky and finally breaks down.

Table I.

Strength of Solubilized Chlorphenol.	"Observed Killing Time."	
	Active *D. dipsaci*.	Revivified "wool" of *D. dipsaci*.
2 per cent.	Practically instantaneous.	1½ minutes.
1 ,,	40 seconds.	3 ,,

At concentrations up to 5 per cent., the solubilizing properties of other benzene derivatives were investigated by the same methods. Figs. 2 and 3 show the relationship of the same detergent to horticultural cresylic acid, a para-meta-cresol mixture and a xylenol.

Chlorination of these compounds renders their solubilization by this type of detergent more difficult ; that is, the detergent : derivative ratio by volume is increased for any given concentration.

McBain (1950) has used X-rays to support his theory of lamellar micelles in explaining this phenomenon. He also gives examples of the way micellar solutions of otherwise insoluble dyes are able to pass through membranes. This property is of great importance when attempts are made to kill soil nematodes by chemical means. By comparing the toxicities of aqueous and solubilized nematicides, some evidence of this property has already been obtained, and it is hoped to present this aspect of the work in due course.

Preliminary Observations on the Effects of Various Chemicals on Stem and Bulb Eelworm (*Ditylenchus dipsaci*).

The discovery that chlorphenol and similar chemicals could readily be solubilized by means of a suitable detergent made it possible to extend the range of toxicity tests considerably ; chemicals could now be tested at concentrations greater than their maximum solubility, without resorting to the use of emulsifiers. It is recognised that, when emulsions are used, the chemicals tend to remain in the surface layers of the soil.

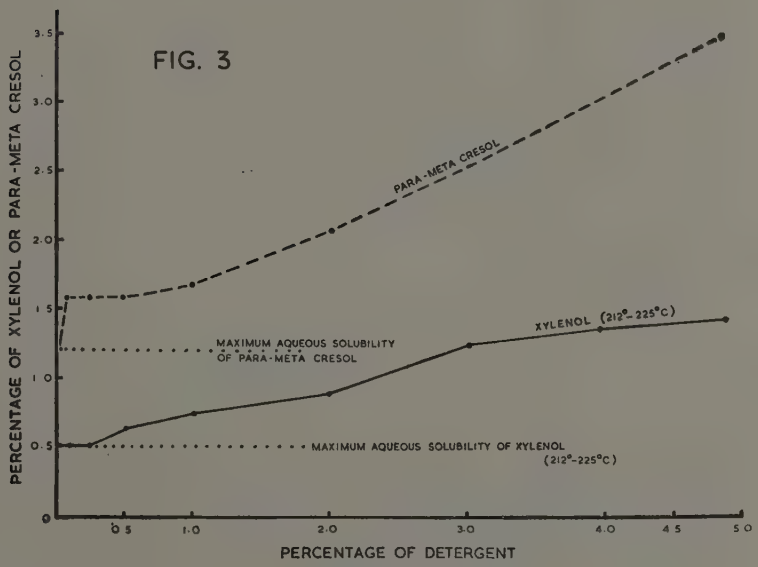

Fig. 3.—Graph illustrating the " solubilization " of para-meta-cresol and a xylenol by means of a detergent.

In order to compare the toxicity of the various chemicals, tests were made using revivified eelworm " wool " of the stem and bulb eelworm (*Ditylenchus dipsaci*). The " wool " stage of this eelworm was used because previous tests had shown it to be more resistant to nematicides, even after it had been revivified, than the active eelworm extracted from plant tissues. The data in Table I illustrate this difference.

The preliminary tests were carried out as follows :—

Eelworm " wool " obtained from a heavily infested stock of narcissus bulbs was revivificd in water. For each test approximately twenty-five worms were placed in a watch-glass and the amount of water was then reduced to a minimum with the aid of a fine pipette. The solubilized chemical was then poured into the watch-glass. The worms were then observed under a binocular microscope until movement had ceased in every individual and this was checked by stimulation of the worms with a needle ; the time taken in this process was then noted and is referred to as the " observed killing time." Several tests were made and the mean of the killing times was finally taken.

Table II sets out the " observed killing time " for a range of chemicals. The strength of the detergent used is given in the second column. Where the amount of detergent is high it is an indication that the particular chemicals, or the particular strengths at which some were used, required more detergent than the others for complete solubilization. As will be seen, the " killing times " range from a few seconds to a period of several hours or more.

Table II.

Chemical.	Strength.	Percentage of Detergent used in Solubilization.	" Observed Killing Time."
Phenol (B.P. 182°C.)	1 per cent.	1 per cent.	20 minutes.
Technical chlorphenol (approximately 70 per cent. ortho-chlorphenol and 30 per cent. di-chlorphenol, 34.5 per cent. chlorine. B.P. 200–212°C.)	2 per cent.	3 per cent.	1½ minutes.
	1 ,,	1 ,,	3 ,,
	0.5 ,,	0.5 ,,	11 ,,
Technical chlorphenol (containing 0.25 per cent. of iodine in addition).	1 per cent.	1 per cent.	40 seconds.
	0.5 ,,	0.5 ,,	1¾ minutes.
	0.25 ,,	0.25 ,,	12 ,,
	0.1 ,,	0.1 ,,	28 ,,
Technical chlorphenol (containing iodine to saturation).	0.5 per cent.	0.5 per cent.	1½ minutes.
Mono-iodo-phenol	1 per cent.	8 per cent.	13 minutes.
Technical chlorphenol (containing 10 per cent. of D-D mixture).	1 per cent.	1.5 per cent.	3 minutes.
	0.5 ,,	0.75 ,,	7 ,,
	0.25 ,,	0.5 ,,	12 ,,
Ortho-chlorphenol	1 per cent.	1 per cent.	4 minutes.

TABLE II (cont.)

Ortho-chlorphenol (containing 0.25 per cent. of iodine).	1 per cent. 0.5 ,,	1 per cent. 0.5 ,,	40 seconds. 2½ minutes.
Technical di-chlorphenol (containing 40 per cent. of chlorine).	1 per cent.	1.5 per cent.	4 minutes.
Technical di-chlorphenol (containing in addition 0.25 per cent. of iodine).	2 per cent. 1 ,,	2 per cent. 1 ,,	35 seconds. 1¼ minutes.
Equal volumes of technical chlorphenol and horticultural cresylic acid.	2 per cent.	3 per cent.	6 minutes.
Horticultural cresylic acid	2 per cent. 1 ,,	3 per cent. 1 ,,	15 minutes. 29 ,,
Horticultural cresylic acid (containing 0.25 per cent. of iodine).	1 per cent.	1 per cent.	13 minutes.
Orthocresol (B.P. 192°C.)	0.5 per cent.	0.5 per cent.	7 minutes.
Para-cresol (B.P. 202°C.)	1 per cent.	1 per cent.	3 minutes.
Meta-cresol (B.P. 202°C.)	1 per cent.	1 per cent.	4 minutes.
Para-meta-cresol mixture 1.50 per cent. (B.P. 202°C.).	1 per cent.	1 per cent.	7 minutes.
Para-chloro-meta-cresol	0.5 per cent.	1 per cent.	4 minutes.
Horticultural cresylic acid (containing 10 per cent. of para-chloro-meta-cresol).	1 per cent.	1.5 per cent.	2 minutes.
A mixture of equal volumes of : (1) Technical chlorphenol containing 10 per cent. of D-D mixture. (2) Technical chlorphenol containing 0.25 per cent. of iodine. (3) Horticultural cresylic acid coning 0.25 per cent. of iodine.	0.5 per cent.	0.75 per cent.	57 seconds.
Xylenol (B.P. 212°–225°C.)	1 per cent.	1 per cent.	8 minutes.
Xylenol (B.P. 209°–212°C.)	1 per cent.	1 per cent.	3 minutes.
Xylenol (B.P. 209°–212°C.) containing 0.25 per cent. of iodine.	1 per cent.	1.5 per cent.	13 minutes.
Mixed chlorxylenols	1 per cent.	7 per cent.	Almost non-toxic. Nearly all active after 5 hours.
Mixed chlorxylenols containing 0.25 per cent. of iodine.	1 per cent.	7 per cent.	Almost non-toxic.

As a result of the solubilization of the chemicals, one fact was at once observed. The solubilization initiated, or increased, the convulsions on the part of the eelworms as the chemicals took effect. The typical convulsions which occur when eelworms are placed in chlorphenol, as compared with the absence of convulsions when iodine solutions are used, has already been recorded.* The convulsions produced by solubilized chlorphenol are much more violent than when an ordinary water solution of the same strength of this chemical is used. This suggests that the entry of the chemicals into the tissues of the eelworms is probably more rapid when the materials are solubilized.

As will be seen later, preliminary toxicity tests made as described above, by recording the " observed killing time " do not always place the chemicals in the correct order. Some chemicals producing a rapid apparent kill of the eelworms are not in fact as toxic as other chemicals which are slower as regards observable action ; but if a chemical gave a poor performance in the preliminary tests it did not prove to be of high toxicity when tested by better methods.

Phenol itself appears to be of low toxicity ; the various cresols and xylenols are of high toxicity. When, however, these compounds are chlorinated the order of toxicity appears to be reversed, i.e. chlorphenol is of high toxicity, as also is chlorcresol, whereas the chlorinated xylenols are of very low toxicity, even lower than that of phenol. Mono-iodo phenol is less toxic than chlorphenol. The toxicity of chlorphenol rises as the percentage of chlorine increases.

It was found that iodine, which is itself highly toxic to eelworms (Staniland, 1950a), is readily soluble in chlorphenol, the various cresols and the xylenols. The addition of a small amount of iodine in this way (0.25 per cent. of the concentrate) appeared to increase the toxicity of the material considerably in the case of the chlorphenols and cresols, but the toxicity is lowered when iodine is dissolved in xylenol or chlorxylenol. The action of iodine, when used in this way, may be synergistic, since saturation of chlorphenol with iodine afforded little increase in toxicity, as compared with the addition of only 0.25 per cent. ; a considerable quantity of iodine can be dissolved in chlorphenol. The comparatively low toxicity of mono-iodo-phenol is also of interest and again suggests that iodine in a combined form has not as great an effect on toxicity as has iodine in solution.

Difficulty was experienced in solubilizing D-D mixture (dichloropropane-dichloropropylene). When, however, the D-D was mixed with chlorophenol in the proportion of 10 per cent. by volume, the mixture

*Staniland (1950).

solubilized satisfactorily ; but the presence of the D-D did not appear to increase toxicity appreciably.

Equal volumes of chlorphenol and horticultural cresylic acid mixed together well and solubilized readily. When used at a strength of 2 per cent. this mixture gave an observed killing time of 6 minutes, which is nearer to the figure for chlorphenol than the mean of the times for each of these materials when used separately at a strength of either 1 or 2 per cent.

Para-chloro-meta-cresol, itself very toxic, was found to dissolve readily in cresylic acid. When dissolved at a concentration of 10 per cent. the resulting mixture, when solubilized at 1 per cent. strength, gave an observed killing time of 2 minutes as compared with 29 minutes for 1 per cent. cresylic acid alone ; the time for 1 per cent. para-chloro-meta-cresol alone is 7 minutes.

Thus, there is in the above mentioned experiments a suggestion that mixtures of chemicals might prove to be more toxic than the single chemicals comprising those mixtures. In particular, there is the example of the mixture of equal volumes of chlorphenol + D-D mixture, chlorphenol + iodine, cresylic acid + iodine and cresylic acid + para-chloro-meta cresol. This mixture gave an observed killing time of 57 seconds when solubilized at 0.5 per cent. This figure of 57 seconds compares most favourably with the figures of 3 minutes for chlorphenol + D-D, $1\frac{3}{4}$ minutes for chlorphenol + iodine, 13 minutes for cresylic acid + iodine (even if this were used at 1 per cent.) or 2 minutes for cresylic acid + chlorcresol (even if used at 1 per cent.) ; if these two latter had been used at 0.5 per cent. their killing times would be considerably longer. Actually each of the four mixtures constituting the collective mixture is only present at a little over 0.1 per cent. and the observed killing time for the most toxic of the mixtures (chlorphenol + iodine) is as much as 28 minutes at that strength.

Controlled Toxicity Tests of Certain Chemicals to Bulb Eelworm (*D. dipsaci*).

In order to obtain some idea of the effect on eelworms of time of exposure to different compounds, a technique was evolved whereby successive batches of eelworms were immersed in the nematicides for a known range of times. The eelworms were then allowed to recover in water.

Technique.

For these experiments, " eelworm wool " obtained from narcissus bulbs heavily infested by *D. dipsaci* was found to be very convenient

as test material. When dry, the eelworms are coiled together in masses; on being wetted, they straighten out, separate, and later become active. Initially, active eelworms from this source were used, but these were found to escape through bolting silk remarkably readily. To obviate this difficulty, the eelworms were used well soaked in water, but before they became active.

FIG. 4

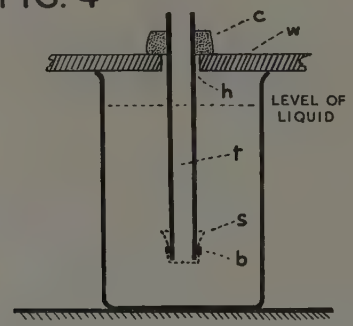

Fig. 4.—Apparatus used in carrying out toxicity tests with " solubilized " chemicals against stem and bulb eelworm (*Ditylenchus dipsaci*).

The apparatus used for carrying out the toxicity tests is shown in Fig. 4. Lengths of $\frac{1}{4}$-inch glass tube, t, 4 inches long, were closed at one end by a layer of bolting silk, s, of 170–190 mesh. The silk was secured by a rubber band, b, made by cutting off a short length of rubber tubing. Each tube was passed through a hole, h, in a wood lath, w, and supported by a cork, c. A convenient length of wood lath is 31 inches having $\frac{1}{2}$-inch holes drilled every $3\frac{1}{2}$ inches, thus handling eight tubes at a time.

Aliquot samples were taken from a suspension of eelworms in a measuring cylinder, and introduced into the tubes, care being taken to place the eelworms on or near the silk. The eelworms in the tubes were exposed to the action of liquids in beakers into which the tubes were lowered. By this means, any desired number of materials could be used at the same time, and all the tubes could be immersed and removed simultaneously. Before the completion of each exposure period, 15 seconds was allowed for draining. The tubes were then moved on together into a series of vessels each containing a large

volume of water, and moved up and down several times to remove any traces of nematicide adhering to the tube and silk. The tubes were then placed in a second rank of vessels containing water. Finally the eelworms were pipetted from the tubes, in water, into solid watch-glasses. Counts of live and dead eelworms were taken when the activity of the control batches had reached a maximum. The worms were viewed through a binocular microscope, and counted between parallel lines scratched on the inner surface of a solid watch glass. Eelworms which were quite motionless, and straight or nearly so, were assumed to be dead; live eelworms were readily picked out by their movement. To assess the kill for each exposure, the figures obtained from each batch were adjusted for mortality in a control tube.

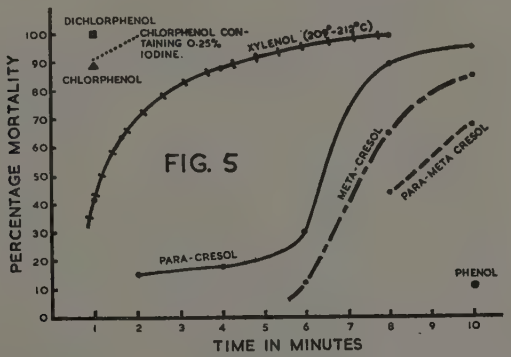

Fig. 5.—The toxicity of various " solubilized " chemicals to stem and bulb eelworm (*Ditylenchus dipsaci*).

Table III gives the results obtained from an experiment of this type using the materials at 0.5 per cent., solubilized by a long chain alkyl sulphate. Tap water was used in making up the solutions, which were at a temperature of 5°C. at the time of treatment. Suitable exposure times were deduced from data obtained from preliminary recovery tests.

It is interesting to note that the mixture of para- and metacresol is less toxic than either material taken separately, as indicated by the preliminary tests. It is also evident that the chlorinated phenols are much more toxic than the other compounds used; this is not evident from preliminary observation figures. It is probable that the

chlorinated phenols are able to penetrate into the eelworms more readily than the others; thus a short exposure enables the eelworms to receive a toxic dose.

TABLE III.

Material Tested.	Time of Exposure in Minutes.	Number of *D.dipsaci* in Sample.	Estimated number originally live. (a)	Number Surviving Treatment.	Number Killed by Treatment. (b)	Per cent. Kill. (b)/(a)	Approximate Time for 50% Kill.
							Mins.
Phenol. B.P.182°C.	10	526	428	383	45	10.5	>10
50 per cent. mixture of para- and meta-cresol. B.P. 202°C.	8	463	377	213	164	43.5	8.5
	10	511	417	132	285	68.4	
Meta-cresol. B.P. 202°C.	6	447	363	319	44	12.1	
	8	507	412	144	268	65.0	7.4
	10	402	327	49	278	85.0	
Para-cresol. B.P. 202°C.	1	262	213	214	—	—	
	2	154	125	108	17	13.6	
	4	195	159	131	28	17.6	6.4
	6	198	161	113	48	29.8	
	8	390	317	36	281	88.6	
	10	524	426	18	408	95.8	
Xylenol. B.P. 209–212°C.	1	262	213	124	89	41.8	
	2	334	272	99	173	63.5	
	4	356	290	35	255	88.0	1.2
	6	413	337	30	307	91.0	
	8	395	322	1	321	99.7	
	10	466	380	7	373	98.1	
Technical chlorphenol 34.5 per cent. cc. B.P. 200–212°C.	1	465	378	41	337	89.2	< 1
Technical chlorphenol containing 0.25 per cent. iodine.	1	264	215	20	195	90.6	< 1
	2	400	376	2	324	99.3	
Technical Dichlorphenol 40 per cent. cc. B.P. 200–212°C.	1 }	Approx.	—	—	All	100	< 1
	2 }	500	—	—		100	

The percentage kills are plotted against time in Fig. 5.

Control: Out of 503 *D. dipsaci*, 409 were alive, or 81.4 per cent.

The Laboratory Treatment of Cysts of Potato Root Eelworm (*Heterodera rostochiensis*) with Nematicides.

This work has comprised the effects of nematicides on eggs containing larvae, the hatched larvae and viable cysts.

Toxicity to larvae.

Active larvae of the potato root eelworm were subjected to the action of solubilized chlorphenol and other chemicals. The tests were carried out in the same way as the preliminary tests described in connection with Bulb Eelworm. The results are shown in Table IV.

TABLE IV.

Chemical.	Strength.	Percentage of Detergent for Solubilization.	Observed Killing Time.
Technical chlorphenol ..	0.5 per cent.	0.5 per cent.	2½ minutes.
,, ,, ..	0.1 ,,	0.1 ,,	32 ,,
Para-chloro-meta-cresol ..	0.5 ,,	0.5 ,,	8 ,,
Technical chlorphenol containing 10 per cent. of D-D mixture	0.25 ,,	0.25 ,,	11 ,,

The above figures indicate that the larvae of this eelworm are easily killed by solubilized chemicals; it was observed that they exhibit the same convulsions as were observed in *D. dipsaci*.

In order to determine whether the solubilized chemicals could penetrate into the cysts, these were removed from moist soil without drying the soil; the soil being heavily infested sufficient viable cysts for this purpose were easily obtained by washing the soil through a fine sieve. Cysts were immersed in 1 per cent. chlorphenol, solubilized with 1 per cent. of detergent; in this solution was dissolved a little soluble blue. Some cysts were removed at varying intervals and washed in water and then transferred to clean water. By pressure on the cysts it was found that the solution containing chemical, detergent and dye had entered the cysts. When pressed a fine stream of dye could be seen issuing from the vulva. Cysts appeared to have become well filled with the dye, in from 10–15 minutes. If left for larger periods the shells of the eggs within the cysts became stained, but no dye was observed to be taken up by the larvae within.

The time required for the chemical to penetrate the cyst, the egg shell, and the larva within the egg, and give a complete kill, therefore lies between 15 and 30 minutes; this bears out the suggestions of the earlier observations.

Eggs were removed from fresh cysts and placed in 1 per cent. solubilized chlorphenol without dye. Observations made under the high power of a microscope disclosed that the larvae began to make convulsive movements after about 10 minutes.

Toxicity to larvae within the eggs.

It seemed, as indicated by the above experiments, that a period in the region of from 15–20 minutes might be sufficient to kill the larvae within the eggs, allowing from 10–15 minutes for the chemical to penetrate through the shell and 2–3 minutes for the killing of the larva, when a 0.5 per cent. solution of chlorphenol was used.

TABLE V.

Treatment with 0.5 per cent. Technical Chlorphenol Solubilized with 0.5 per cent. Detergent for periods of :	Eggs Hatched by 13th August.
3 minutes.	Eggs mostly hatched but not as many as from the control.
6 ,,	Moderate hatch.
9 ,,	Small numbers hatched.
12 ,,	Small numbers hatched.
15 ,,	Only three eggs hatched.
30 ,,	No eggs hatched.
60 ,,	No eggs hatched.
Control (untreated).	Nearly all the eggs hatched.

In order to test this, batches of several hundred eggs were removed from cysts ; any larvae found already free were removed. The water around the eggs was drained with a very fine pipette and solubilized chlorphenol (0.5 per cent. with 0.5 per cent. of detergent) was added to each watchglass. A control sample was kept in water. The chlorphenol was removed at varying intervals from the different batches of eggs, the eggs then being washed thoroughly with water and left in the clean water for 3 days. The water was then replaced by potato root diffusate and the hatching of the larvae was recorded. The untreated eggs were found to hatch freely under these conditions. The tests were carried out on 17th July, diffusate added on 20th July and larvae commenced to hatch on 23rd July. The experiment continued until 13th August by which date a large proportion of the untreated eggs had hatched, the larvae being very active. The results are shown in Table V.

A number of eggs which had not hatched, in all the treatments, were opened and the larvae freed. The condition of the larvae proved to be of great interest.

At the end of the experiment, i.e. 21 days after treatment, there were no observable differences by which it was possible to distinguish dead eggs from those still alive. Signs of discolouration and a definite breakdown of larval contents of treated eggs have been observed in other experiments, but after a much longer period from the date of treatment. When the unhatched eggs were opened it was at once

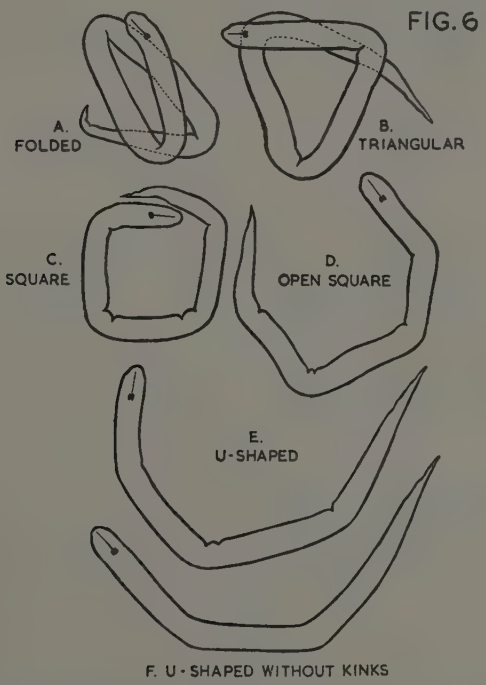

Fig. 6.—Diagram illustrating the various formations taken up by dead larvae of the potato root eelworm (*Heterodera rostochiensis*) when removed from the eggs.

apparent that dead larvae could readily be distinguished from live larvae. The chemical appears to coagulate the protoplasmic contents of the larvae and possibly hardens the cuticle ; the result is that the larvae from treated eggs do not spring out readily when the shell is ruptured, as always happens with live larvae. The dead larvae emerge in a variety of formations and their condition may, in general, be described as "kinked."

A range of the various formations of the larvae is shown in Fig. 6, and the type of formation, as will be shown later, bears a relation to the strength of the chemical used and on the period which has elapsed since the treatment was given.

The larval formations may be described as follows :—

A. "*Folded.*" The larva has expanded very little and is still folded up much as it was in the egg.

B. "*Triangular.*" In the example shown the tip of the tail has straightened out, but often this is found conforming to the triangle.

C. "*Square.*"

D. "*Open Square.*"

E. "*U-shaped.*" There are various stages between a "Close U" and a "Shallow U."

In all the formations A–E it will be observed that there are definite "kinks," or corrugations of the cuticle on the inner surface of the larvae. These kinks do not disappear on keeping the larvae in water, unless they are only very slight ; and the kinked larvae only alter their formation slightly with the passage of time.

F. This shows the formation of a larva when a live egg from a well-dried cyst is opened. The larva at first emerges as a "shallow U," but without kinking. After a short while in water the larva becomes straight. When a well-soaked healthy cyst is examined the larvae at once come out straight.

The method provides a useful check on hatching tests with root diffusate and for estimating the toxicity of chemicals on occasions when it is not possible to use diffusate. Larval examinations are clearly best carried out after the cysts have been well soaked in water. Attempts are being made to devise a technique for the rapid opening of batches of eggs so that larval formation may be placed on a numerical basis.

Examination of the unhatched eggs from the batches of treated and untreated eggs referred to in Table V, showed that they were largely or entirely in the form of triangles or squares ; this confirmed the results given by the hatching tests.

Experiments in the Treatment of Cysts.

Experiments were now conducted to see if the larvae enclosed in the eggs could be killed without removing them from the cysts ; cysts were recovered from infested soil by the usual flotation methods and were soaked in water before treatment. A batch of 50 cysts was

treated in 0.5 per cent. Technical Chlorphenol (with 0.5 per cent. detergent) for a period of 1 hour and then washed with water. A similar batch were kept for the same period in water. Hatching tests with diffusate were then carried out over a period of 4 weeks. From the control a total of 965 larvae hatched, but no larvae emerged from the treated eggs. Batches of 50 cysts were also treated for 1 hour with 1 per cent. horticultural cresylic acid (with 1.5 per cent. detergent) and with 1 per cent. para-meta-cresol (with 0.5 per cent. detergent). The control cysts were kept in water for the same period. The hatch with root diffusate between 15th September and 13th October was 5 larvae from the cresylic acid, 9 larvae from the para-meta-cresol and 267 from the untreated cysts. Unhatched eggs from the treated cysts were opened and the larvae were nearly all kinked. From the untreated cysts a few larvae were kinked.

Experiments in the Treatment of Cysts mixed with Soil.

Further experiments were carried out to determine if a control could be obtained when soil containing cysts was treated. In the first instance, soil was treated in a manner which would be similar to a commercial treatment in a glasshouse.

A quantity of moist soil known to be heavily infested with *H. rostochiensis* was mixed thoroughly and placed into two waxed 5-inch pots. The soil was in its natural state, in fairly fine tilth ; it was firmed down in the pots.

Into one pot, 300 ccs. of 0.5 per cent. chlorinated phenol, solubilized with detergent were poured on to the soil surface through a fine tube, so that the material could be distributed as evenly as possible. The other pot received 300 ccs. of water. After allowing excess liquid to drain from the pots, they were wrapped in damp cloths to prevent the soil drying out, and allowed to remain for one calendar month.

After this period it was noticed that the soil soaked in water only had a thick layer of fungal hyphae on the surface. No hyphae were visible on the treated soil. Each soil was then air-dried and the contained cysts extracted by the usual flotation method. The cysts were soaked in water overnight, and were then arranged into batches of 50 in solid watch-glasses. Cysts for the first batch of 50 from each soil were deliberately chosen with maximum viability in mind. Selection was easier from the untreated cysts, since the ones with the greatest egg content had sunk in the water overnight. The treated cysts, however, seemed to have lost their capacity for imbibing water and very few had sunk. Egg masses were visible through the cyst

walls and, in this case, visual assessment was necessary to supplement sunken cysts to make up the batch. The remaining cysts from each soil were arranged into batches of 50 at random. There were sufficient cysts for 5 further batches of 50 in each case, making 12 batches in all; 6 batches of cysts obtained from treated soil, and 6 from untreated soil.

The 12 batches of cysts were then submitted to the action of potato-root diffusate. The intention was to record the hatch from the 2 batches of selected cysts at frequent intervals. The hatch from the other 10 batches was to be collected and total hatches estimated by aliquot methods after hatching had ceased.

TABLE VI.

Condition of Soil.	Strength of Technical Chlorphenol.	Percentage of Detergent used for Solubilization.	Treatment and Length.	Number of Larvae Emerging.
Dry	0.5 per cent.	0.5 per cent.	2 minutes.	Nil
			1 hour.	,,
			24 hours.	,,
	—	—	Control (water only for 24 hours).	3,438
Moist ..	1 per cent.	1 per cent.	1 hour.	Nil
	0.5 ,,	0.5 ,,	2 minutes.	233
			1 hour.	11
	—	—	Control (water only for 1 hour).	404
	0.25 per cent.	0.25 per cent.	30 minutes.	4
			1 hour.	2
	—	—	Control (water only for 1 hour).	132
	0.1 per cent.	0.1 per cent.	2 hours.	14
			3 ,,	13
			4 ,,	3
	—	—	Control (water only for 4 hours).	139

Hatching from the batches of untreated cysts was considerable. From the selected batch, the total hatch was 5,670 larvae after 55 days. Fenwick's (1951) shortened method gave an estimated 50 per cent. hatch of 2,900 corresponding to a log-time of 0.9—an error of less than 5 per cent. As was expected, the hatch from the other untreated lots of cysts was less than this. The counts were estimated and varied between 500 and 2,000.

No hatching at all took place from any of the cysts recovered from the treated soil. The cysts were opened afterwards and the contained eggs were seen to be aggregated and blackened.

Under laboratory conditions, therefore, there can be no doubt of the efficacy of this type of soil treatment.

Shorter treatments of infested soil were now made. The amount of soil used in each case was 50 grams, the soil being heavily infested. The experiments were conducted with soil which had become almost dry in the laboratory and with other soil which had been kept out of doors and was in a naturally moist condition. The soils were treated for varying periods and with several strengths of solubilized chlorphenol; samples of soil both dry and moist were treated with water only for the same period as the longest chemical treatment. The treatments were made by placing the soil in a funnel on filter paper and pouring on the chemical. In this way all surplus liquid drained from the soil. The soil was not sieved or specially broken up, but was as collected on the field, where there was a reasonably good tilth.

Hatching tests were carried out on the cysts floated from the soils, after they had been spread out thinly so as to dry quickly. On the average, over 250 cysts were recovered from each 50 grams of soil. The hatching tests were carried out over a period of 30 days. The results are shown in Table VI.

In these experiments it was realised that the drying of the soils after treatment, though quick, was not removing the chemical and that a gradual concentration of the chemical might also be taking place. A further experiment was therefore carried out to determine whether an immediate and thorough washing of the soil at the end of the treatment would be reflected in the results. At the time the experiment was made no root diffusate was available and the results were assessed by examination of larvae freed from the eggs.

A duplicate series of 50-gram lots of moist soil was treated for 2 hours in funnels as before; technical chlorphenol was used in strengths ranging from 5 per cent. down to 0.1 per cent., with the appropriate amount of detergent necessary for solubilization. Untreated controls (water only for 2 hours) were included. One set of soils was thoroughly washed, as soon as the treatment was over, with graded sieves. The fine silt and cysts retained on the fine sieve were then spread out thinly to dry and the cysts recovered by flotation in the usual way. The soils in the other series were not washed, but spread out thinly to dry and the cysts recovered. The cysts from the two series were then stored dry in corked tubes for a period of 20 days

so as to allow time for any larvae which had been killed to become set and exhibit kinking when removed from the eggs.

Ten full cysts were examined from each sample of treated soil, washed and unwashed, and a good proportion of these eggs were opened. The kill of larvae was complete in the soils treated with 5, 3, 1, 0.5 and 0.25 per cent. chlorphenol in the case of both washed and unwashed soils. It was of interest that the majority of the larvae came out folded from both the soils treated at 5 per cent., whereas those treated at 3 per cent. were mostly of triangular formation ; the remaining treatments were rather variable, but there was a preponderance of U-shaped larvae displaying kinking. Only about one third of the larvae were dead in the cysts treated at 0.1 per cent. The untreated controls when opened gave U-shaped larvae without kinks and after a short time these straightened out, but did not display any movement.

The remaining cysts were kept dry for two months and then soaked in water for 3 days. Cysts from each treatment in both series were then examined in the same way. Larvae from the untreated cysts came out straight and many displayed movement. Larvae from the cysts treated at 0.1 per cent. came out straight in the proportion of about one third, and many of these also displayed movement. The larvae from the remaining treatments were kinked, but tended to drop one category as regards formation, i.e. folded larvae tended to give place to larvae with triangular formation—triangles to squares—and so on down the series ; but the treatments of 0.25 per cent. and upwards for 2 hours appeared to have given a complete control and there was no indication that washing of the soils and cysts had reduced the efficacy of the treatments ; it suggested that, once the chemical had filled the cyst and entered the egg, washing would not easily remove it.

A test was also made of 1 per cent. para-meta cresol (with 0.5 per cent. detergent) ; the soil, being treated for 1 hour on 22nd October, dried quickly and the cysts extracted and placed in water. A month later it was clear that a complete kill had been obtained, the extracted larvae being almost all in triangular formation.

Experiments on the Wetting of Soil by Liquids.

While work on this important aspect of the problem has not yet been pursued in detail, sufficient has been done to stress its importance and to indicate the direction in which further work is required.

In the first instance movement of the liquids was observed in dry

and moist sand which was placed in glass cylinders. A 0.5 per cent. solution of the detergent was prepared and in this was dissolved sufficient fuchsin to produce a deeply dyed fluid. Enough liquid was added to saturate the top inch of the dry sand. A similar amount was then poured on to the moist sand, which was just damp enough to cling together.

In the dry sand the dyed liquid formed a clean band of colour, 1 inch deep. In the damp sand the coloured area extended downwards a little over 3 inches, the lower part being less deeply stained than the upper portion. After a few days the stained layer in the dry sand had moved slightly downwards but the edge was still well defined. In the moist sand there had been greater and more diffuse movement downwards. A similar quantity of water was now added to each cylinder. The dyed layer in the dry sand moved clearly down by that amount and left the sand above almost unstained; the lower edge was still clean and sharp. In the moist sand there was a further diffuse extension downwards and the sand at the top became as clean as with the dry sand.

The time required for penetration of 350 ccs. (the equivalent of 10 gallons per square yard) of 0.5 per cent. detergent and 1 per cent. cresylic acid into cylinders of damp soil was determined; the cylinders were 4 inches in diameter and 14 inches deep and were made of waxed paper; the soil was 12 inches deep. The progress of the liquid down through the soil could be gauged by pressing the sides of the cylinder with the fingers, when the position of the wet and more compressible soil could be felt. The progress of the movement of the liquid was as follows:—

0 minutes—The liquid was trickled on evenly and steadily from a fine tube.

1 minute —Ponding of the liquid on the surface commenced.

6 minutes—All the liquid had been poured on and now "ponded" 1 inch deep.

30 minutes—Surface just becoming free of "ponding."

36 minutes—Soil wet down to 9 inches.

45 minutes—Soil wet to 10 inches.

65 minutes—Still about 10 inches.

90 minutes—Soil wet down to 12 inches.

As a check on penetration, cysts were recovered from the soil 16 days later and a sample of these cysts was opened and the larvae examined; they were found to be in triangular and square formation.

Preliminary investigations indicated that the higher surface tension of water causes it to run more rapidly through the soil capillaries and does not wet the soil particles thoroughly. The lower surface tension of the detergent solution causes it to be retained more in the soil. To obtain an estimate of the difference in the degrees of retention, glass cylinders were filled with dry soil in a fine and in an average state of division; both soils were packed tightly in the cylinders. Since 350 ccs. of water was known to wet such cylinders of soil completely, 400 ccs. of water, and the same volume of 1 per cent. detergent, were added to the various cylinders, care being taken to prevent the liquids running down the inside surfaces. The amounts of surplus liquids which ran away at the bottom of the cylinders was measured. Where detergent was used approximately 10 per cent. less ran away.

The soils were examined afterwards and it was found that the water above had not penetrated the larger soil lumps, while the detergent had done so and was clearly remaining in the soil. Where detergent was used the soil was of a spongy texture and easily compressible; the soil wetted with water was much harder and tended to retain its original structure. The relation of surface tension to the efficiency of soil nematicides in solution is clearly important and is being examined further.

Since approximately 10 gallons of liquid per square yard is required to wet the top 12 inches of an average soil thoroughly, some attention was given to the problem of reducing the amount of liquid, if possible. The quantity of 10 gallons per square yard is feasible in commercial glasshouse practice; glasshouse growers are accustomed to treating their soil with heavy applications of water. Under outdoor conditions, however, such a practice would be difficult. It was thought possible that solubilized chemicals could be applied at greater strength, but in smaller volumes, to the surface of soil; rain falling over a period might then be relied upon to dilute the solubilized chemical and distribute it in depth.

To test this theory waxed paper cylinders, of the same size as before, were filled to a depth of 12 inches with tightly packed soil, moist and of fairly fine tilth. On 20th September, 100 ccs. of a 5 per cent. cresylic acid (solubilized with 7 per cent. of detergent) was trickled on to the surface. Starting on the following day, and continuing at intervals of a few days until 17th October, 250 ccs. of water were also trickled on—approximately 50 ccs. on each occasion; by 17th October the cylinder had therefore received total liquid equivalent to 10 gallons per square yard. The bottom inch of soil was removed

by cutting through the paper and soil with a sharp knife. Cysts were recovered from this bottom inch of soil ; examination of larvae removed from many eggs from a number of cysts showed them to be dead and mostly in triangular formation. This showed that the washing in, and dilution, of a strong solubilized chemical by rain could offer a practical means of reducing the quantity of liquid applied to the soil.

The solubilization of a high percentage of a chemical, of the order of 5 per cent., requires a higher concentration still of detergent ; on dilution the percentage of detergent would be higher than is required. It was thought, therefore, that a compromise might prove equally satisfactory ; this was to use, in making the 5 per cent. solution, that amount of detergent which would be used in a full volume treatment, i.e. 5 per cent. cresylic acid and 2.5 per cent. detergent. On dilution to one tenth of the strength this would provide a solution of 1 per cent. cresylic acid solubilized with 0.5 per cent. of detergent.

The result of such a mixture is that the cresylic acid is partly solubilized and partly emulsified. It had already been found that an emulsion applied to sand was converted into a dilute solubilized form when leached out with water and that the leachings were toxic to eelworm. Soil in a waxed paper cylinder was treated with the emulsified cresylic acid and again washed down over a period with water. Cysts were examined and it was again found that many of the larvae were dead, though the kill was rather less than when the fully solubilized chemical was diluted.

Experiments in the Field.

Small pilot experiments based on the laboratory results were carried out against potato root eelworm on tomatoes under glass and an experiment was included on potatoes in the open.

(a) *Glasshouse experiments.*

Two experiments were carried out in Somerset.

Centre I.

At this centre the viable cysts content of the soil was of the order of 5 viable cysts (600 eggs) per gram.

The treatments consisted of 0.5 per cent. solubilized chlorphenol, 1 per cent. solubilized horticultural cresylic acid and untreated plots ; the plots were duplicated. Treatments, at the rate of 9 gallons of liquid per square yard were carried out on 15th February. Planting was on 26th March, after formaldehyde treatment by the grower. The soil was very light and dusty in character.

At this centre the plants on all the treated plots had clearly suffered a check as the result of soil treatments. (It should, however, be stated that this was the only centre of five, where tomato soils were treated, in which any ill effects were to be seen.) Later, the plants on the treated plots recovered and subsequently made much better growth than those on the untreated plots. Despite this extra growth, the plants on the treated plots exhibited a yellowish-green colour as compared with the darker green of the control plants. The plants on the chlorphenol plots were better than those on the cresylic acid plots.

Cyst and egg counts were made on all the plots before and after treatment; hatching of larvae was also carried out by means of root diffusate; but the results were inconclusive. However, the plants in the treated plots, particularly those receiving chlorphenol, were much better than those in the controls, cropped more heavily and, in particular, made exceptionally good root growth. The roots on the control plants were very small in number and severely damaged; those on the treated plots were extensive, white and free from rotting, though scattered cysts could be found.

Centre II.

At this centre the viable cyst content of the soil was of the order of 3 cysts (300 eggs) per gram.

The treatments consisted of iodine used at a strength of 1 in 8,000 (with the necessary potassium iodide for solution); a second plot received this same iodine treatment with the addition of 0.5 per cent. of detergent. Two plots were treated with 0.5 per cent. of chlorphenol (the concentrate of this contained 0.25 per cent. of iodine); two plots were untreated.

The treatments were again made at the rate of 9 gallons per square yard on 22nd and 23rd January. Tomatoes were planted on 10th March, after formaldehyde treatment by the grower. The soil was a medium loam.

No phytotoxic effects were observed at this centre. It was apparent soon after planting that the plants on the plots treated with chlorphenol (containing iodine) were making very rapid growth as compared with all the other plants. The iodine plots, with and without detergent, were indistinguishable from the controls.

The plants on the chlorphenol plots again exhibited yellowish-green foliage as compared with all other plots.

The plants on the control plots soon ceased to grow and made practically no fresh roots outside the original ball; these roots were

in a very seriously rotted condition. The roots of the chlorphenol treated plants were extensive, white and very healthy in appearance.

Roots were examined on the control and chlorphenol plots on 14th May and 26th June. On 14th May no cysts were visible on the chlorphenol treated plants, though subsequent laboratory examinations showed scattered individuals within the tissues in the form of second and third stage larvae. White cysts were very plentiful on the roots of the control plants. By 26th June there were scattered white cysts on the chlorphenol plants; but brown cysts were very plentiful on the control plants. These observations suggest the possibility of some retarded hatching (of the order of some 40 days) having occurred, as a result of the treatments, in addition to the killing of cysts.

The subsequent growth of the plants on the chlorphenol plots was of the order of five times the height of the control plants. The grower, in an endeavour to improve the condition of the plants on those plots not treated with chlorphenol, mulched the plants heavily, but with very little result. This procedure made it impossible to complete the experiment in the manner intended. No cyst counts or hatching tests were possible except on the chlorphenol plots, which the grower did not touch. Cyst counts made on these plots before and after treatment are of interest. The counts were :—

	0.5 per cent. strength chlorphenol (containing 0.25 per cent. iodine in the concentrate).
Plot I.	*Viable Cysts per 50 grams.*
Before treatment ...	179.0
At end of season ...	99.5
Plot II.	
Before treatment ...	111.0
At end of season ...	64.5

(b) *Experiments on Potatoes in the field.*

A small series of plots were laid down in the Bromham district of Wiltshire; the soil is light and sandy.

The viable cyst content was of the order of 15 per 50 grams (40 eggs per gram).

The treatments consisted of :—

(1) 5 per cent. chlorphenol (solubilized with 7 per cent. of detergent) at the rate of 1 gallon per square yard.

(2) 0.5 per cent. chlorphenol (0.5 per cent. detergent) at the rate of 10 gallons per square yard.

These plots were duplicated, as also were the controls. Applications were made on 25th April and potatoes of the variety Majestic were planted on 25th May.

No phytotoxic effects were seen on the plots receiving the high volume chlorphenol (0.5 per cent. strength). Experiments to ascertain the safe interval for planting potatoes after thorough soaking of the soil with 0.5 per cent. solubilized chlorphenol were carried out elsewhere on a similar light soil. Potatoes were planted immediately before the treatment of the soil and at three-day intervals after the treatment, the variety being Majestic. No damage occurred to tubers planted nine, or more, days after the soil treatment, but it occurred with the earlier plantings.

On the plots treated with low volume chlorphenol (5 per cent.) at Bromham, there were a few gaps and a few plants slightly weakened, but growth was not in general adversely affected.

In the beginning there was little difference in rate of growth on the various plots, but later on the high volume (0.5 per cent.) chlorphenol plots were the best, followed closely by the low volume plots.

By 16th August the amount of root on the plants on the treated plots was markedly greater than that of the plants on the control plots. No cysts could be found on samples of treated roots which were examined, but brown cysts could readily be found on the control plots.

The plots were lifted on 21st September and the yields were as follows :—

Low volume (5 per cent.) chlorphenol...	(a) 15 lbs. (b) 25 lbs.	Mean =20 lbs.
High volume (0.5 per cent.) chlorphenol	(a) 25 lbs. (b) 30½ lbs.	Mean =27¾ lbs.
Control	(a) 15 lbs. (b) 20 lbs.	Mean =17½ lbs.

The plots were resampled after lifting. A comparison of the egg counts before treatment and after lifting showed that on the control plots the figure had increased between 2½ and 3 times ; on the low volume (5 per cent.) chlorphenol plots the increase was between 1½ and 2 ; on the high volume (0.5 per cent.) the increase was also between 1½ and 2.

The plots have been treated again (17th October) and observations will again be made next season.

Potatoes from the two treatments and from the controls were cooked and a tasting test was carried out. Of five tasters, only one

individual appeared to be able to detect any tainting and in this case it occurred on potatoes from a low volume (5 per cent.) plot ; but the taint was admitted to be very slight. No tainting could be detected by anyone on the high volume (0.5 per cent.) samples.

The Tainting of Tomatoes.

A special problem has arisen with the discovery that chlorphenol can taint tomato fruits strongly. Two instances occurred where tomatoes indirectly became tainted in this way. In the first case, chlorphenol was being used in bulb baths some hundred yards away from tomato houses on the same holding. The tomatoes developed a strong taint. There seemed no possible way by which drainings from the bath could have got into the houses, or into the water supplies used for the watering of the plants. Eventually the mystery was solved by finding that a leaking container of chlorphenol had been stored in a shed where tomatoes packed for market remained overnight before being consigned. The second case was of a bulb bath used in the open close to a glasshouse. The vapour from the bath evidently entered the ventilators of the house and caused the tainting.

Five soil treatments with chlorphenol have been carried out in glasshouses, of which two were concerned with potato root eelworm and have been described in this paper. In one of these two cases definite tainting occurred and in the other there was indirect evidence that it probably also took place. In both these glasshouses absorbent brick walling was present and it is thought probable that the chlorphenol was absorbed by these walls and given off as a vapour later on, when the temperature of the houses rose. The chlorphenol in these houses was applied by means of a hose and the brickwork was wetted. In the three houses where no tainting occurred, there were either no absorbent walls near the plants or the brickwork was not wetted, the chlorphenol being applied carefully to the surface of the soil with a watering can.

The suggestion that tainting is probably by vapour and not by absorption of the chemical from the soil is strengthened by these three cases of freedom from taint ; also by the fact that where tainting did occur it was present on other plants in the house where the soil had not been treated. There is also the case in the West Midland Province (communicated by letter) where Newton treated a series of potted plants and reported tainting of the fruit ; the porous pots probably stored up the chlorphenol and later released it in vapour form.

A treatment carried out by Thomas in Devon (also communicated by letter) is of special interest. The chlorphenol was applied in July, when the rest of the houses was not only planted, but was bearing green and partly coloured fruit. The chemical did not get on the walls and this again strongly suggests that the plant does not take up chlorphenol from the soil, since no trace of tainting occurred throughout the season.

The use of chlorphenol when tomatoes are grown must therefore be approached with extreme caution. It is possible that nematicides other than chlorphenol will have to be used for this purpose.

Ripe tomatoes were suspended in sealed vessels in which were placed quantities of the various chemicals. After 24 hours the fruits were tasted, with the following results :—

Concentrated Technical Chlorphenol ...	Very severe tainting.
0.25 per cent. Solution of Chlorphenol ...	Fairly severe tainting.
Concentrated Xylenol (212°–225°C.) ...	No tainting.
,, ,, (209°–212°C.) ...	Very slight tainting.
,, para-meta-cresol	No tainting.
,, Horticultural Cresylic Acid	Very slight tainting.
Solid para-chloro-meta-cresol	No tainting.
0.5 per cent. Solution of Para-chloro-meta-cresol	No tainting.

Discussion.

Previous work (Staniland, 1950) showed that chlorphenol, in ordinary water solution, up to 0.5 per cent. strength was highly toxic to eelworms, in particular *D. dipsaci*. It was, however, found that chlorphenol in solution was of variable toxicity to the cyst stage of the potato root eelworm, *H. rostochiensis*, though of sufficient promise to warrant further work.

The use of chlorphenol and related compounds in micellar solution, i.e. " solubilized," has given markedly better results and has widened the field of use of such compounds very considerably, and this paper deals with those aspects of the problem. By solubilization of the chemicals with a detergent of the long-chain alkyl sulphate group, the materials can now be used in a soluble form at strengths greater than their maximum normal solubility ; in addition, they can be used in a form in which they are readily carried through membranes, such as egg shells and nematode cuticles, by the action of the solubilizing detergent.

The power of the solubilized chemicals to wet and penetrate into soil is greater than with ordinary solutions and it is probable that the

chemicals are also rendered more persistent in the soil; this persistence, however, is not so great as to bring phytocidal problems to the fore, as is shown by the fact that potatoes can be grown satisfactorily as early as 9 days after the soil has been thoroughly soaked in 0.5 per cent. solubilized chlorphenol.

The laboratory experiments which have been described show that nematicides of the types tested, and in the manner used, are highly toxic to nematodes; it is clear that there exists a wide range of similar compounds still awaiting test. The ones already tested are effective at relatively low concentrations even against the cyst stage of potato root eelworm.

While the work, at the stage it has reached, cannot be regarded as an economic answer to all problems of eelworm control, with the possible exception of the treatment of glasshouse soils, it nevertheless brings into prominence a new approach to the control of nematodes, i.e. control by contact using nematicides in a form capable of penetrating membranes.

Future development of the work aims at :—

(1) Toxicity tests of a wide range of chemicals lending themselves to solubilization.

(2) A study of the movement of liquids, and of solubilized chemicals in particular, in soil. The conditions for reaching a high proportion of eelworms in the soil, as suggested by the work, are :—

   (a) The soil should be as dry as possible.
   (b) The soil should be as fine as possible.
   (c) The soil, having the above characteristics, should be well compacted.

The practical application of liquids to soil is being investigated. The correct conditions for application of liquids to soil are of special importance in connexion with eelworms having a cyst stage; where eelworms are moving freely in the soil the problem is likely to be easier.

In addition to a study of the problem of reaching the cysts in soil, there is also the problem of effecting this with the smallest possible amount of liquid. It has already been shown that there is a possibility of doing this by the dilution of a small volume of solubilized chemicals by rain. The efficiency of such methods could be considerable if further chemicals are found of even greater toxicity than those already described, which will also lend themselves readily to solubilization.

Solubilized chemicals have shown themselves capable of bringing about a very marked increase in top and root growth, coupled with increased cropping. It is suggested that these effects are due to the control of eelworm and not to the well-known "soil amendment" effect. The increased growth has been accompanied by a yellowish-green colour of the leaves which, far from suggesting any increase in available nitrogen, points rather to a temporary shortage of plant food, possibly as the result of inhibition of some of the nitrifying bacteria in the soil.

Summary.

1. The treatment of clover and teazle seeds with solubilized chlorphenol has been carried out and found to be equally harmless to the seeds, as regards germination, as the ordinary solutions previously described. The mixing of the micellar solution, and its use, is described.

2. The use of chlorphenol in bulb baths for the treatment of narcissus bulbs infested with eelworm is reviewed in the light of the solubilization of chlorphenol. The strength of chlorphenol is reduced from 0.25 per cent. to 0.1 per cent., the percentage of detergent remaining at 0.25 per cent. Mixing of the solution and its use, are described.

3. Advice is given on the handling of chlorphenol.

4. Details are given of the solubilization of several benzene derivatives by means of detergents of the long-chain alkyl sulphate type.

5. Preliminary observations on the effects of various chemicals on the stem and bulb eelworm (*Ditylenchus dipsaci*) are described. Phenol is of low toxicity, cresols and xylenols of greater toxicity ; the order is, however, reversed when these compounds are chlorinated, i.e. chlorphenols are the most toxic and chlorxylenols the least.

6. Controlled toxicity tests of certain of the chemicals referred to in (5) show that, of those tested, the chlorphenols are the most toxic but that other chemicals are also of high toxicity.

7. Evidence is put forward to show that solubilized chemicals can penetrate into cysts and through the egg shells into the larvae. The various formations exhibited by dead larvae when freed from the eggs are illustrated and discussed.

8. Experiments on the treatment of cysts of potato root eelworm extracted from soil have confirmed that solubilized chemicals can effect a complete kill.

9. The experiments with cysts have been repeated on soil containing cysts and similar results have been obtained.

10. Experiments relating to the wetting and penetration of soil by solubilized chemicals are described and the salient points of this problem are discussed.

11. Experimental methods of reducing the volume of solubilized chemicals applied to the soil are described and it is shown that such procedures may be feasible.

12. Small pilot plots in the field using solubilized chemicals against potato root eelworm on tomatoes under glass and on potatoes in the field, are described. In all cases increased growth and yield resulted and increased rooting took place. The behaviour of the eelworm population is not yet clear, though there is evidence at some centres of a reduced rate of increase with some treatments.

13. Tainting of tomatoes occurred at some centres and this problem is discussed.

14. The general results of the work are discussed, at the stage which it has now reached.

ACKNOWLEDGMENTS.

Acknowledgments of the help of the Agricultural Research Council and of Dr. H. H. Eley have already been made elsewhere in this paper. Thanks are also due to Messrs. British Alkaloids Ltd. of London, and to Messrs. Wm. Butler and Sons, Ltd. of Bristol, for kindly supplying samples of various chemicals and for help in the preparation of various compounds to meet the requirements of the work.

The authors also wish to record valued assistance by various members of the Advisory Entomologist's Department at Bristol N.A.A.S. Headquarters, and in particular by Miss A. Pain, and to various growers for permission to carry out experiments on their holdings.

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Journal of Helminthology, Vol. XXVII, Nos. 1/2, 1953, pp. 75—80

Intestinal Infestation of Turkey Poults with *Plagiorchis (Multiglandularis) megalorchis* Rees, 1952 and an Experimental Study of its Life-Cycle.

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In 1937 Foggie described an outbreak of necrotic enteritis in turkey poults in Northern Ireland. This outbreak, in which twenty-two out of twenty-three poults died, was caused by a small trematode, provisionally identified by Foggie as *Plagiorchis laricola* Skrjabin. This appears to be the only record of infestation of turkey poults with a trematode of the genus *Plagiorchis*.

The disease in Radnorshire.

On June 8th, 1950, four turkey poults, two weeks of age, were received for routine autopsy. They had been dead 36–48 hours at the time of post mortem examination and were in fairly good condition and of average size. The only significant lesion, shown by all four poults, was that throughout the length of the small intestine the mucosa appeared necrotic and detached from the sub-mucosa. As putrefaction was occurring it was impossible to determine whether this was an ante mortem necrosis or a post mortem change or both. The lower third of the small intestine contained food material and several hundred trematodes, the latter being chiefly found on the lumenal surface of the detached mucous membrane. Scrapings from the caecal mucosa and sub-mucosa indicated coccidial infection but, as few oocysts were observed and the caecal wall was apparently undamaged, it was not thought to be of much significance.

The identification of the parasite

The trematode was named *Plagiorchis* (*Multiglandularis*) *megalorchis* by Dr. G. Rees (1952) of the Department of Zoology, University College of Wales, Aberystwyth, who found it to be identical with that described and provisionally identified by Foggie as *P. laricola*. But until Skrjabin's type material and description become available for comparison it is impossible to identify Foggie's or Rees' specimens as *P. laricola*.

The epizootiology and history of the Radnor case.

The poults examined had been hatched and reared on a South Radnorshire farm, situated at an altitude of approximately 600 feet. The environs within a radius of five miles varied from 600–1,500 feet. There was much marsh land and rough pasture on the farm and district and also numerous streams and large ponds. Mixed farming was practised and the poultry were hens, ducks, geese and turkeys. Because of the apparently rare occurrence of this trematode in turkeys it is reasonable to assume that this bird is not the normal host. Amongst the the wild birds seen in the farm and district were wild duck, curlew, plover, and black headed gull and it may be possible that *Plagiorchis(M.) megalorchis* normally occurs in one of these. It is interesting to note that *P. laricola* was recorded by Skrjabin from gulls and terns.

A small number of turkeys are reared annually on the premises. In the spring of 1950 there was only one hen turkey ; she laid ten eggs and eight were purchased and added to these to make a sitting. From this sitting the turkey hen hatched eighteen poults, two of which were killed accidently at a day old. At two weeks of age thirteen of the remaining sixteen poults died within a fifteen hour period, and four of these (mentioned above) were received for autopsy. A week after the deaths had occurred the farm was visited (June 15th, 1950). The three survivors, now three weeks of age, were stunted in growth and unevenly feathered. The turkey hen was apparently unaffected throughout as also were the other poultry on the premises. At this time the poults were the only young poultry stock on the farm and for this reason may have been the only susceptible poultry.

The hen and poults had been, and the survivors still were, housed in a dark and dirty shed with a mud floor. They were on free range and had access to the farm yard where effluent from the stable and cattle houses, and overflow from a metal drinking tank drained into a muddy pond in one corner of the yard. The pond overflow seeped under a stone wall onto a pasture field adjacent. This pond was probably the only source of drinking water for the poults. At the time of the visit the pond was drying and was represented by a number of isolated puddles in the mud. Each puddle was teeming with insect larvae, namely *Chironomus riparius*, and two species of *Culicoides*, which at the time appeared to be possible second intermediate hosts of the trematode. No gastropod mollusc which might prove to be the first intermediate host could be found in this pond. However about fifty yards from the pond and running along the adjoining pasture field was a brook in which were found numerous specimens of the fresh water

molluscs *Lymnaea pereger* and *Ancylus fluviatilis*. Specimens of the various insect larvae and water snails were brought back and examined by Rees for larval stages of *P.* (*M.*) *megalorchis*. Rees found such trematode larvae in large numbers of the *L. pereger* examined and also made the important observation that it was possible to infect the insect larvae found in the farm yard pond with cercariae emerging from the snail.

At one month of age, one of the surviving three poults was removed from the farm, and on arrival at the laboratory was killed and autopsied. The only abnormality observed was a mild rickets. The intestine and contents were examined but no evidence of enteritis or trematodes could be found. In December, 1950, approximately six months after the deaths in the poults the turkey hen was slaughtered for the Christmas market and the intestines were examined but no trematodes or any intestinal abnormality were found.

Investigation into the life cycle of the trematode.

Because of the observations made by Rees mentioned above it was decided to investigate further the nature of the developmental stages seen in the snail and insect larvae and to attempt the infestation of a definitive host. Although the turkey is probably not the natural final host of *Plagiorchis* (*M.*) *megalorchis*, it was decided to use it for experimental infestation because the parasite had been found to infest poults. For this purpose six, day old, bronze turkey poults hatched on July 3rd, 1950, were obtained from an accredited breeder. From hatching until used for experiment these poults were housed in a large wire cage, the floor of which was covered with grease proof paper which was changed daily. The cages were in a building which precluded the possibility of contamination of food, water, or the cages themselves by other birds or poultry. The water was taken from the main supply and the food consisted of proprietary chick pellets incorporating cod liver oil, and also chick grit.

First Experiment.

On July 7th, 1950, two poults were selected at random and isolated from the remaining four. In the two groups the housing, feeding and management were identical apart from the experimental infection of the two isolated birds. These birds were wing banded No. 1 and No. 2 and treated as follows :—

Poult No. 1 was given 50 infested larvae of two species of *Culicoides* and 8 infested larvae of *Chironomous riparius*. The larvae had previously

been experimentally infested with cercariae of *Plagiorchis* (*M.*) *megalorchis*. This work was undertaken by Rees throughout the experiment. The heavy worm infestation had killed most of the larvae, which were administered to the poult by pipette into the mouth. By 24 hours this poult was depressed and disinclined to feed. It became progressively weaker and on the fifth day (July 11th) it was too weak to stand. It died and was autopsied on this date.

Poult No. 2 was treated similarly to poult No. 1. Infestation was over three successive days on each of which the poult was given 50 infested larvae of two species of *Culicoides*. Most of the larvae had been killed by the heavy cercarial burden. This bird appeared normal throughout the experiment and was killed and autopsied on the sixth day (July 12th).

The autopsy on both birds included a gross examination of all the organs and tissues and an examination of the mucous membrane of the small intestine and the small intestinal contents. For this the intestine was ligated at the junction with the gizzard and caecal tubes, removed from the carcase, and under normal saline opened and examined with a dissecting microscope. An aerobic bacteriological examination was made from the heart blood, liver and lungs onto blood agar and McConkey agar.

Poult No. 1 was found to be emaciated but no macroscopic abnormality was otherwise seen. Poult No. 2 was in good condition and no gross abnormality was observed. No trematodes were found in the small intestine or contents of either poults, and for both the bacteriological examinations were negative.

Second Experiment.

As it was probable that the insect larvae, used in the first experiment, had been killed by the mass penetration of cercariae before encystment could occur and, further, since flukes generally do not develop in the final host unless encystment in the second intermediate host is complete, it was decided to feed insect larvae in which encysted cercariae were present. In the second experiment, therefore, an attempt was made to feed insect larvae in which cercarial encystment was complete. Two more poults were taken from the remaining four and isolated as in the first experiment. They were wing banded No. 3 and No. 4 and were 17 and 18 days old respectively when experimental infection was commenced, July 20th and 21st, 1950.

Poult No. 3 was given on July 20th, 70 living insect larvae, each containing about 10 encysted cercariae. The cysts were about six

days old. On July 24th it was fed 90 living insect larvae, each containing about 10 encysted cercariae composed of three groups roughly equal in numbers and containing respectively cysts of three, four, and five days old.

Poult No. 4 was given on July 21st, 40 living insect larvae each containing about ten cercarial cysts, some of which were three and others four days old.

Both birds ate the larvae from a Petri dish and appeared in good health throughout the experiment. On July 29th, poult No. 3 was killed and examined. The condition of the carcase was good and no macroscopic abnormality was observed. In the small intestine between 14 and 18 inches distant from the gizzard five sexually mature trematodes were found. One appeared to be attached to the mucous membrane and the others were free in the lumen amongst the intestinal contents. There was no macroscopic enteritis. These trematodes were identified by Rees as identical with those found in the original poults, namely *Plagiorchis* (*M.*) *megalorchis*. On July 30th, poult No. 4 was killed and immediately autopsied. The carcase was in good condition and the only gross abnormality seen was in incrustation of the mucous membrane of the crop from which *Candida albicans* was isolated. Between the 16th and 24th inch of the small intestine four sexually mature trematodes were observed free in the lumen and again found by Rees identical with *Plagiorchis* (*M.*) *megalorchis*. No enteritis was seen.

The two control poults had grown normally and one of these was killed on August 1st, 1950, at 29 days old and an autopsy made. No abnormalities and no trematodes were found in the intestine.

Symptoms of disease did not appear in the experimental birds No. 3 and No. 4 probably because the infestation was not sufficiently heavy and the poults were in better condition and older than those dying apparently of fluke infestation on the Radnorshire farm.

SUMMARY.

1. Heavy intestinal infestation with *Plagiorchis* (*M.*) *megalorchis* Rees, 1952, in four two-week old turkey poults is described. This was presumably the cause of death in the four poults and possibly of nine other birds of the same hatch.

2. The epizootiology of the condition is described.

3. The life-cycle of the parasite has been investigated, using turkey poults as the definitive host.

ACKNOWLEDGMENTS.

The help and guidance given by Dr. G. Rees, Department of Zoology, University College of Wales, Aberystwyth, is gratefully acknowledged.

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Journal of Helminthology, Vol. XXVII, *Nos.* 1/2, 1953, *pp.* 81—94.

On Certain Eelworms, including Bütschli's *Tylenchus fungorum*, obtained from Toadstools.

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In one of the early classical works on free-living nematodes Bütschli (1873) described certain forms which he placed in the genus *Tylenchus* Bastian, 1865. One of them he named *Tylenchus fungorum* and gave an account of the morphology of the adults which are depicted in his Figs. 11a–c, Tafel II. The male tail, as represented in Figs. 11b and 11c, has an unusually voluminous bursa surrounding the tail tip whilst the spicules are extraordinarily large and quite unlike those of any other known species of *Tylenchus*. Each spicule possesses an elongated posterior process lying outside the cloacal slit. Ventral post-anal papillae are also figured.

So far as the writer is aware this peculiar nematode has not been seen again since Bütschli's day and apart from attempts by various systematists (including the present writer) to establish its taxonomic status, based entirely on Bütschli's original work, no further description or drawings of it embodying fresh observations have been published since 1873.

Bütschli obtained his examples of the nematode from decaying fungi (" faulenden Pilze ") but gave no indication of the genus and species in which they occurred.

The writer had the good fortune in the late autumn of 1951 to receive samples of eelworms which had been collected from rotting basidiomycetous fungi. Amongst them were several males and females of this species as well as those of a new species belonging to the same genus. From the detailed study of these nematodes it soon became apparent that the females possess a gonad of the type found in young adult females of the insect-infesting genera *Allantonema* and *Tylenchinema* in which a rudimentary ovary is joined by an oviduct to a more or less capacious uterus which, after copulation, becomes crowded with spermatozoa. Bütschli, however, figured as the adult female of his *T. fungorum* a nematode with a distinct oesophagus and a well-developed gonad having eggs about to escape from the vulva. This, in the writer's opinion, could not possibly be the true female corresponding to the male.

All the females examined by the writer had a rudimentary ovary and the uterus densely packed with spermatozoa which agreed in shape with those in the vas deferens of the males. One was therefore driven to the conclusion that these nematodes are not to be regarded as forms which complete the life cycle in the free state. The females must, in fact, become parasitic in the body-cavity of some insect or other, as in the case of *Allantonema* and *Tylenchinema*, for the completion of their development. At the time of writing there has been no opportunity for putting this hypothesis to the test and one must wait for the re-appearance of the requisite toadstools and the insects breeding therein before further observations can be made.

Meanwhile it seems desirable to publish an account of the morphology of the adult males and females of the two species found. They do not belong to the genus *Tylenchus* as now understood but to the genus *Iotonchium* Cobb, 1920. The two species herein described are *Iotonchium fungorum* (Bütschli, 1873) n. comb. and *Iotonchium bifurcatum* n. sp.

A discussion on the taxonomy of the nematodes is given later.

Material.

The writer is greatly indebted to Prof. P. A. Buxton, C.M.G., F.R.S., Director of the Department of Entomology, London School of Hygiene and Tropical Medicine, for his kindness in sending the eelworms on which the observations have been made. In studying insects occurring in various kinds of toadstools, Prof. Buxton uses a technique whereby the fungal material is placed in wide-mouthed glass jars, the bottoms of which are covered with a layer of damp sawdust. Each jar is provided with a special stopper in the centre of which is a disc of wire gauze to allow aeration. Under these conditions eelworms have been found, often in considerable numbers, in the condensation moisture on the inside of the jars whence they have been removed on small pieces of damp filter paper and sent to the writer in corked specimen tubes.

The tubes first received on 20th November, 1951, were from the three following fungi, (1) *Entoloma rhodipolium*, (2) *Hygrophorus virgineus*, (3) *Tricholoma cunifolium*. *I. fungorum* and *I. bifurcatum* were present in good numbers in the collection from *Entoloma rhodipolium*. Along with them were numerous examples of a *Rhabditis* species and large numbers of *Rhabditophanes schneideri* (Bütschli, 1873) n. comb., syn. *Cheilobus quadrilabiatus* Cobb, 1924 ; a species also first found by Bütschli (1873) in rotting fungi and described by him under

the name *Rhabditis schneideri*. The eelworms from *Tricholoma cunifolium* and *Hygrophorus virgineus* consisted solely of *Rhabditophanes schneideri*.

Later in November, 1951, further nematodes were received from a jar in which a specimen of *Pleurotus cornucopiae* had been placed. These consisted of larvae of a *Rhabditis* species. In January, 1952, two further collections were sent by Prof. Buxton, one from a jar containing *Pleurotus corticatus* and the other *Pleurotus ostreatus*. Both of these contained spermatized females of *I. fungorum*, that from *P. corticatus* furnishing a single male example and several females whilst the eelworms from *P. ostreatus* consisted of females only. Males, however, must have been present in the original culture medium as all the females had been spermatized.

Methods.

A preliminary examination of the eelworms was made in tap water after relaxation by heat when the chief anatomical features of both sexes were made out. Many specimens were collected, carefully killed by heat at 65°C. and then fixed in a formal-acetic mixture consisting of formalin 40 per cent., 10 ml., glacial acetic acid, 10 ml., water, 80 ml. In subsequent microscopical study many specimens were examined mounted in this fixative and permanent mounts were made in lacto-phenol cotton blue [see Franklin, M. T. and Goodey, J. B. (1949) and Goodey (1951)] and in glycerine.

Morphology.

Iotonchium fungorum (Bütschli, 1873) n. comb.

syn. *Tylenchus fungorum* Bütschli, 1873, pro parte.

Hexatylus fungorum (Bütschli, 1873) Goodey, 1932, pro parte.

Male: 0.94 to 2.15 mm., a = 40–50 ; b = ? ; c = 15–22.

Average of 11 1.73 mm. ; a = 46.5 ; b = ? ; c = 18.2.

As the measurements show there is a considerable range in size, some males being under 1 mm. and others 2 mm. or more long. The cuticle carries rather fine transverse striations which encircle the dorsal and ventral surfaces of the body but end at the edges of the lateral fields which extend from the head to the beginning of the bursa (Fig. 1, a and d). Each field is between $\frac{1}{4}$–$\frac{1}{3}$ the width of the body and carries two inner lines which begin close to the head but increase to 4 and sometimes 6–8 towards the tail as shown in Fig. 1, d. Each field tapers anteriorly with the forward tapering of the body. In the centre of each in the region of the hemizonid, or a little in advance of it, is a deirid.

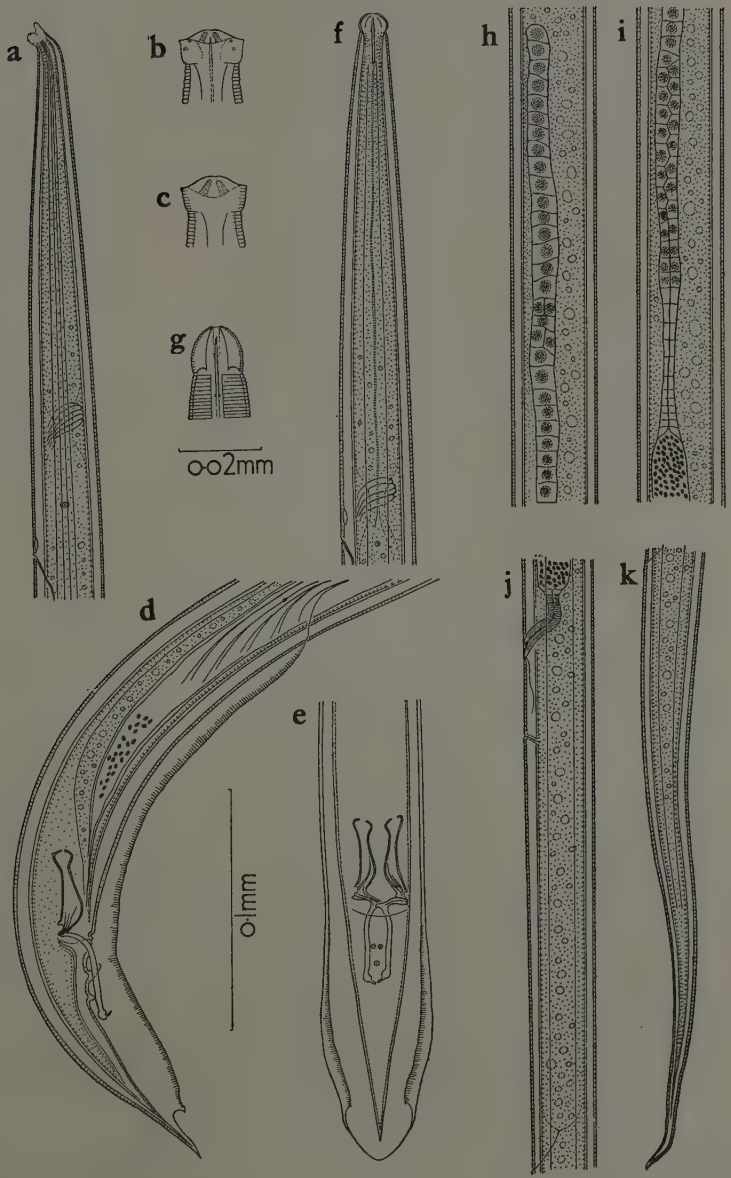
The hemizonid lies immediately anterior to the excretory pore and in true lateral aspect has the appearance of a flattish lens. In his original description of *I. fungorum*, Bütschli speaks of the transverse striae on the body and the presence of lateral fields but does not bring out the shape of the head of the male ; he merely states that he found traces of lips.

The head, which bears transverse striae on its dorsal surface, has the same shape and structure as that of the male of *I. imperfectum* figured by Bütschli, being dorso-ventrally flattened so as to produce two rather blunt, squarish lobes each bearing two small papillae, one of which is almost medially situated on the ventral face and the other near the anterior edge. Dorsally the two lobes are connected by an anteriorly convex ridge and ventrally by a thin anteriorly concave membrane. A clear stoma is lacking but in the mid-ventral line there is a shallow depression in which the sharp point of the inconspicuous spear is located. The posterior part of the spear is but weakly developed and is practically invisible. A faint lumen traverses the oesophagus which is poorly defined and lacks muscle fibres. Only in the region of the nerve ring is it possible to detect an indication of a constriction of the tissues which gives rise to a rather more clearly defined line of separation between the oesophagus and the body wall. Posterior to the level of the nerve ring the oesophagus blends imperceptibly with the intestine which stretches backwards into the bursal region where it narrows down and unites with the vas deferns to form a common cloacal duct.

The testis is single and outstretched ; the anterior end, in well developed specimens, lying a short distance behind the level of the excretory pore. The vas deferens, which occupies the bulk of the organ, is rather wide and is crowded with the small lens-like sperms which are produced in enormous numbers. The bursa is very large ; the wings arising ventro-laterally from the edge of the lateral field more than five times the anal body width in advance of the cloacal opening. The free edge of each wing bears fine transverse striae. The tip of the tail is completely enclosed by the bursa which is deeply recessed ventro-

Fig. 1.—*Iotonchium fungorum* (Bütschli, 1873) n. comb., a-e male ; f-k female. a, anterior region in lateral view ; b and c, head in ventral and dorsal view respectively ; d, tail in lateral view ; e, tail in ventral view ; f, anterior end and oesophageal region in lateral view ; g, head, more highly magnified in lateral view ; h and i, region of ovary, oviduct, and forepart of uterus with sperms ; j. posterior end of uterus, vagina, vulva, post-vulval mid-ventral supplement and anus ; k, anus to tail tip. Scale between d and e applies to all, except b, c and g to which the scale immediately under g applies.

FIG. 1



laterally just in front of the plain dorsal lobe surrounding the tail tip. The spicules are paired (see Fig. 1, d and e). Their characteristic shape was depicted in lateral and ventral view by Bütschli. Each consists of two main parts, viz., a fairly stout anterior portion (a little more than 30 μ long) the head of which is offset by expansion, and a narrow posterior part. The shaft of this anterior part narrows posteriorly to form a laterally directed elbow with a strengthening ridge which curves outwards from the inner surface of the shaft. From the elbow each spicule bends sharply inwards and forwards towards the mid-ventral line where the two have the appearance of being fused together. Real fusion, however, is not effected but merely an overlap. Each spicule then tapers to the second part, about 30 μ long, the greater part of which lies outside the transverse cloacal slit, and has the appearance of a narrow tube lying on either side of the mid-ventral line. The posterior end of each limb expands slightly to form a knob and then tapers to a short finger-like process directed inwards. A gubernaculum is absent. There is a pair of post-anal ventral papillae located one on either side of the mid-ventral line about halfway between the cloaca and the ends of the posterior parts of the spicules. An additional single mid-ventral post-anal papilla is situated a short distance behind this pair. The first pair are prominent when the male tail is seen in lateral aspect.

Female: 2.77 to 3.70 mm., a=75–93; b=?; c=12.0–14.2; V=81–84% Average of 13 specimens 3.04 mm., a=85; b=?; c=13.4; V=83%.

Bütschli gave 3 mm. as the length of the female *Tylenchus fungorum*; a length agreeing closely with that determined by the writer. Nevertheless the nematode depicted in his Fig. 11a is not the female of *Iotonchium fungorum*. The chief reasons for the writer making this apparently dogmatic statement are that the eelworm is shown with a well developed gonad, eggs filling the uterus and ready to pass out of the vulva which is situated close to the rather broad end of the body only a short distance in front of the anus. It is fruitless to speculate as to the identity of the female shown by Bütschli beyond suggesting that it probably belongs to the writer's *Hexatylus* or to a closely related genus. A description is therefore given of the females found by the writer. The body is for the most part cylindrical but tapers slightly anteriorly from the level of the excretory pore and posteriorly from the region of the vulva to the sharply pointed tail. The cuticle has fine transverse striae which encircle the dorsal and ventral surfaces of the body but not the lateral fields which extend from close to the head almost to the tail tip. No incisures or longitudinal markings have been

found on the lateral fields but, as in the male, a deirid is present on each field at about the latitude of the excretory pore. A hemizonid occurs just in front of the excretory pore.

The head is offset by a slight constriction. It is dome-shaped and bears six ridges which divide the surface into 6 sectors (Fig. 1, g). These lines are visible under the oil-immersion but only on favourable specimens. There are indications also of inner bars or struts connected with the lower ends of the ridges which show that there is a head framework resembling, in general features, that found in many tylenchid eelworms. Very fine transverse striae also appear to be present on the head.

The stoma is terminal and leads into a small vestibule in which lies the anterior part of the mouth spear. The latter is 15 μ long and is composed of two parts, viz., a conical anterior region about 5 μ long with an oblique aperture and a posterior shaft about 10 μ long, the base of which carries small lateral thickenings.

As in the male, the oesophagus is very poorly delimited from the body wall but shows a constriction within the nerve ring. By very careful focussing it has been possible to discern faint indications of a lumen but no signs have been found of any lateral ducts or inlets opening in to the lumen from oesophageal or intestinal glands such as occur in *Tylenchinema*, *Allantonema* and *Heterotylenchus*. It is reasonable to suppose, by analogy with these forms, that such glands and accompanying ducts may be found to occur in species of *Iotonchium* and possibly they may be located when an opportunity has occurred of studying larval development in these nematodes. The intestine calls for no special description. It stretches back in the body and is connected by the rectum to the anus.

The gonad consists of four principal regions, ovary, oviduct, uterus and vagina. The ovary is single and is outstretched. In one specimen drawn under oil immersion it consists of a column of more than 50 cells, first in single and then in a double array ; the whole measuring about 0.3 mm. in length. The oviduct is rather narrower than the ovary, is 0.065 mm. long and is made up of smaller cells arranged on either side of a narrow lumen. It expands a little posteriorly where it joints the uterus. The thin-walled tubular uterus measures 0.91 mm. and extends backwards to its junction with the vagina. In most of the specimens studied the uterus was more or less completely filled with small spermatozoa which are lens-like or ovoid in outline with a denser rod-like centre. The vagina is about 30 μ long, has stout muscular walls and a narrow

lumen. It is rather S-shaped and directed posteriorly where it opens at the transverse vulva. The cuticle forming the anterior lip of the vulva covers the vulval aperture and extends on to the ventro-lateral body surface for a short distance ; its attachment to the body being marked by a distinct line. In the mid-ventral line, at about 37 μ behind the vulva, there is an innervated supplement traversing the body wall. It has been found in all the female specimens examined by the writer.

Occurrence. Sexually adult males and spermatized females occurred in the following basidiomycetous fungi, *Entoloma rhodipolium*, *Pleurotus corticatus* and *Pleurotus ostreatus* ; all at Gerrards Cross, Bucks., England.

Type specimens. No original holotype is available for this species; the author has therefore selected a neotype and paratypes which are deposited at the Nematology Department, Rothamsted Experimental Station, Harpenden, Herts.

Iotonchium bifurcatum n. sp.

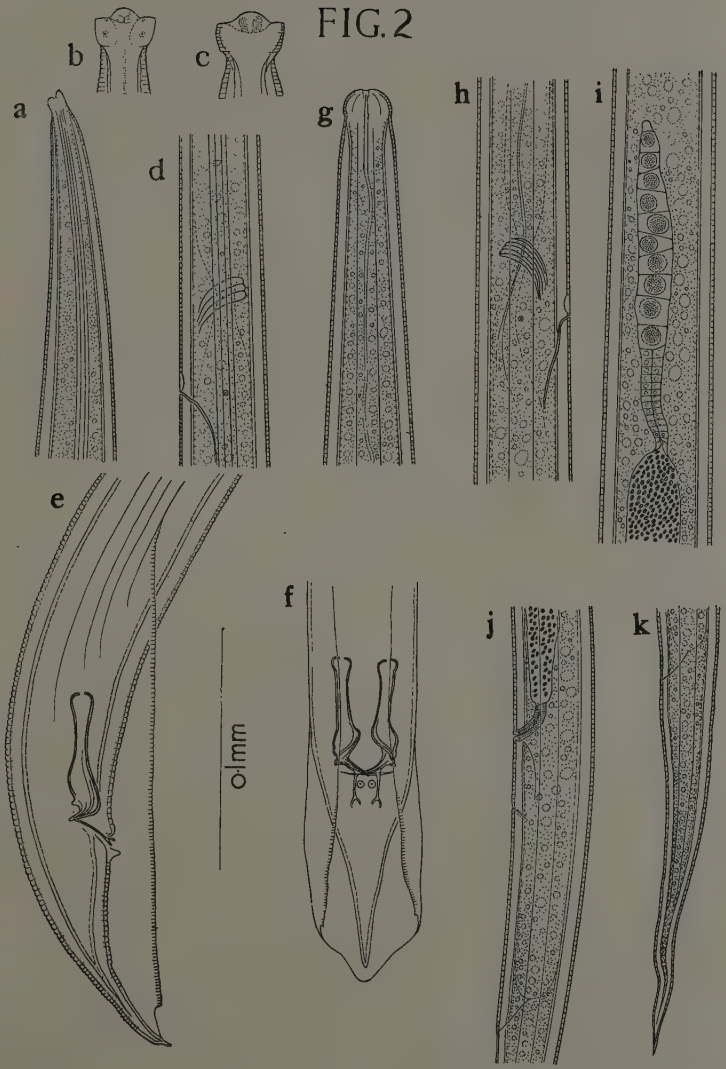
Male: 1.0 to 1.49 mm., a = 30–40 ; b = ? ; c = 17.5–23.6.

The body is slightly arcuate ventrally when relaxed by heat. It tapers towards the anterior end. The cuticle carries fine transverse striations on the ventral and dorsal surfaces ; the striae being interrupted at the lateral fields which extend from the head to the beginning of the bursal wings (Fig. 2, a, b and e). Each field carries two longitudinal incisures which divide the field into 3 areas, all being approximately of the same width throughout. A deirid is located on each field at about the same level as the excretory pore ; its papillate core lying in a shallow depression at the centre of a circular disc. A lens-like hemizonid occurs immediately in front of the excretory pore.

The head has the same peculiar shape as that found in males of *I. fungorum*. When examined from the dorsal or the ventral position under high magnification, it is found to consist of two lateral lobes, rather rectangular in outline, which are connected dorsally by a thickened, anteriorly convex ridge and, ventrally, by a thin anteriorly concave membrane. On the ventral side each lateral lobe carries two

Fig. 2.—*Iotonchium bifurcatum* n. sp., a-f, male ; g-k, female. a, anterior region, lateral view ; b, head, ventral view ; c, head, dorsal view ; d, posterior oesophageal region showing nerve-ring, hemizonid, deirid and excretory pore ; e, tail in lateral view, termination of gonoduct and intestine omitted ; f, tail in ventral view ; g, anterior region showing head and mouth spear ; h, posterior oesophageal region ; i, ovary, oviduct and anterior end of uterus containing sperms ; j. posterior end of uterus, muscular vagina, vulva, post-vulval mid-ventral supplement and anus ; k, tail region. All at same magnification except b and c, to which the .02 mm. scale in Fig. 1 applies.

FIG. 2



small papillae ; one almost medially situated and the other located near the anterior edge (Fig. 2, c and d).

There is a small shallow depression in the middle line in between the dorsal and ventral connecting ridges, which must be regarded as the mouth opening though a well defined stoma and vestibule are lacking. In this lies the small sharply-pointed fore part of the mouth spear. The posterior tubular part is either lacking or is so weakly developed as to be indiscernible. The lateral lobes narrow a little posteriorly where they blend with the ventral surface of the body so that the head is slightly offset by expansion. Transverse striae have been seen on the dorsal side of the head. There appears to be an inconspicuous lumen leading backwards from the region of the spear into the oesophagus. The latter is not sharply defined and lacks muscle fibres. Where the nerve ring encircles it, however, there is some indication of a constriction of the oesophageal tissues. The intestinal region calls for no special mention. The testis is single and outstretched anteriorly. The bulk of the gonad consists of the rather wide vas deferens which becomes crowded with small lens-like spermatozoa each with a central rod-like core.

The bursa is comparatively voluminous and surrounds the tip of the tail (Fig. 2, e and f). Each bursal wing arises from the ventral edge of the lateral field, the lines of which splay out on to the forward half of the wing. On either side of the body, just before the terminal dorsal lobe, each wing is recessed ventro-laterally. The free edge of each wing carries fine lateral striations. Phasmids or lateral caudal papillae have not been seen even under high magnification. There is, however, a pair of large conical post-anal ventral papillae, just behind the cloacal slit, one on either side of the mid-ventral line.

The spicules are large and each consists of a rather stout shaft about 30 μ long the head of which is slightly expanded. The narrower middle portion, about 10–12 μ long, then swells again towards the lower end. The inner face curves over ventrally as a stout ridge to produce an elbow and then tapers inwards almost at right angles towards the middle line where the two spicules overlap (Fig. 2, f). These tapering pieces lie just within the transverse cloacal aperture through which the final narrow process of each spicule is exerted. Each process, which is about 10 μ long, is provided with a bifurcate tip, a feature on which the specific name has been erected.

Female: 1.20 to 1.51 mm., a=50–60; b=?; c=18.6–15; V=81–84%.

The body tapers a little anteriorly and considerably posteriorly from just in front of the anus to the sharply pointed tail. The cuticle bears

fine transverse striae which end at the plain lateral fields stretching from just behind the head practically to the tail tip. A deirid is located at the centre of each field at the latitude of the excretory pore, immediately in front of which is the hemizonid. The head is rounded in front and is offset by a very slight constriction. It carries six faint ridges which form a delicate head framework and divide it into 6 sectors. The mouth is terminal and central leading into a short vestibule in which lies the point of the mouth spear. The latter is 20 μ long and is made up of an anterior conical part about 5.5 μ long followed by a posterior shaft 14.5 μ long, without basal thickenings or swellings. The oseophagus is very indistinct and is only recognisably marked off from the body wall where it is constricted in passing through the nerve ring. A very faint lumen traverses its length and leads into the beginning of the intestine. The latter extends throughout the body and is connected with the anus by a faint rectum. The gonad is single and outstretched anteriorly. It is made up of four chief regions; ovary, oviduct, uterus and vagina. The ovary consists of a short column of cells each with a large nucleus. The number of component cells varies from 5 to 10 (Fig. 2, i). Behind the ovarian primordium is the oviduct composed of smaller cells which in optical section can be seen to line a fine central lumen leading into the front end of the uterus. This is a thin-walled tube extending backwards in the body until it connects with the muscular vagina. In all the specimens examined it was crowded with spermatozoa, the latter having the same shape and size as those in the males. The vagina is ventrally arcuate. It opens on the ventral surface at the rather small transverse vulva. The cuticle forming the anterior lip of the vulva extends backwards over the vulval aperture and its attachment to the body posterior to the vulva can be seen as a line on the ventro-lateral surface of the body. At about 20 μ behind the vulva, in the mid-ventral line, there is an innervated supplement which traverses the body wall.

Occurrence. In rotting specimens of the basidiomycetous fungus, *Entoloma rhodipolium* collected at Gerrards Cross, Bucks., England.

Type specimens. Holotype—male; allotype—female and paratypes in the Nematology Department, Rothamsted Experimental Station.

TAXONOMY.

The systematic relationships of the nematodes just described call for some further consideration and discussion. In particular it is desirable to establish the genus *Iotonchium* Cobb, 1920, and amend it to embrace the two additional species which are now assigned to it,

In erecting *Iotonchium* Cobb put forward the combination *Iotonchium imperfectum* (Bütschli) nom. nov ; at the same time citing *Tylenchus imperfectus* Bütschli as a synonym. The new name was accompanied by a brief description of the nematodes ; in effect an abbreviated translation of Bütschli's original description without the study of new material. The combination so set up effectively established *Iotonchium* as a monotypic genus and, according to Article 30 (c) of the Rules of Zoological Nomenclature, *I. imperfectum* of necessity becomes its type species.

It must be pointed out, however, that the alleged female of the species described by Bütschli cannot be the true female corresponding to the male since it is figured with a segmented egg in the vagina. The shape of the male head, the voluminous bursa and the characteristic L-shaped spicules reveal, however, the close kinship of the males to those described earlier in this paper and there is no doubt in the writer's mind that all three species should be placed in one and the same genus. The genus *Iotonchium* is, therefore, formally defined and amended so as to include the three species.

Order : Tylenchida Thorne, 1949

Superfamily : Tylenchoidea Chitwood & Chitwood, 1937

Family : Allantonematidae Chitwood & Chitwood, 1937

Subfamily : Iotonchinae nov. subfam.

Genus : *Iotonchium* Cobb, 1920 (emend.)

Type species : *Iotonchium imperfectum* (Bütschli, 1876) Cobb, 1920

syn. *Tylenchus imperfectus* Bütschli, 1876.

Definition. Males with dorso-ventrally flattened lobate head hollowed anteriorly. Mouth spear weakly developed in males, oesophagus ill-defined, non-muscular. Bursa voluminous. Ventral post-anal papillae present. Spicules large, markedly angular and L-shaped ; in two species with posterior extensions capable of protrusion or permanently extruded through cloacal opening. Gubernaculum absent. Females with well-developed mouth spear and poorly defined non-muscular oesophagus. Female gonad consisting of rudimentary ovary, short oviduct, tubular uterus and muscular vagina ; the uterus after copulation serving as a receptacle for spermatozoa. Lateral fields, deirids and a hemizonid present in both sexes.

Other species : *Iotonchium fungorum* (Bütschli, 1873) n. comb. syn. *Tylenchus fungorum* Bütschli, 1873 ; and *Iotonchium bifurcatum* n. sp. *Iotonchium zeae* Sch. Stek., 1941 and *I. obtusicauda* Sch. Stek., 1941 are

both excluded from the genus *Iotonchium* as just defined. They should, in the writer's opinion, be assigned to the genus *Neotylenchus* Steiner, 1931, or to some nearly related genus.

Occurrence. In the free state in various species of basidiomycetous fungi; the sexually-ripe, gravid female probably living as a parasite in the body-cavity of some fungus-inhabiting insect.

Discussion. A few remarks are needed on the genus in relation to the higher categories set out above. It is placed in the order Tylenchida Thorne, 1949 because of the character of the mouth spear in the female; in the superfamily Tylenchoidea Chitwood & Chitwood, 1937 because of the occurrence in both sexes of deirids and a hemizonid; in the family Allantonematidae Chitwood & Chitwood, 1937 because of the absence of oesophageal muscles and the features of the female gonad. Finally a new subfamily Iotonchinae is proposed for its reception because of the unusual shape and structure of the male head, the remarkably large L-shaped spicules, the voluminous bursa, the presence of post-anal ventral papillae in the male and a post-anal mid-ventral supplement in the female.

SUMMARY.

1. A detailed morphological study has been made of certain nematodes occurring in the basidiomycetous fungi, *Entoloma rhodipolium*, *Pleurotus corticatus*, *P. ostreatus*, *Hygrophorus virgineus* and *Tricholoma cunifolium*.

2. From the first three of these, males and females of two species of eelworms have been obtained which are placed in the genus *Iotonchium* Cobb, 1920. One of these is *Iotonchium fungorum* (Bütschli, 1873) n. comb., originally described by Bütschli under the name of *Tylenchus fungorum*; the other is a new species which is named *I. bifurcatum* n. sp.

3. The males of both species have peculiar lobed, dorso-ventrally flattened heads and a poorly developed mouth spear. The bursa is very large, the spicules have posterior prolongations which are extruded through the cloaca and ventral post-anal papillae are present. A gubernaculum is absent.

4. The females have normal dome-shaped heads, possess a stoma and a well-developed mouth spear. The gonad consists of ovary, oviduct, uterus and vagina as found in females of *Allantonema* and *Tylenchinema* forms which, after spermatization, become parasitic in the body-cavity of insects. It is suggested that the females of the

two species under consideration must ultimately become parasitic in some fungus-inhabiting insect.

5. The taxonomic position of the new genus is discussed. A new subfamily, Iotonchinae, is erected which is placed in the family Allantonematidae, in the order Tylenchida Thorne, 1949.

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Journal of Helminthology, Vol. XXVII, Nos. 1/2, 1953, pp. 95—103.

An Investigation into the Method of Dispersal of *Panagrellus silusiae,* with Particular Reference to its Desiccation Resistance.

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Panagrellus silusiae was first described by de Man (1913) from specimens found among the fauna of the so-called "Bierfilzen," on which beer mugs used to be placed, in German inns. Aubertot (1925) records having found the same worm at Strasbourg in 1923, from traps containing fermenting potato puree which he had set to catch *Drosophila*. He believed that the flies had brought the eelworms. Allied species have been recorded from various media where bacterial or fungal activity has produced organic acids. *P. redivivus* (Linn., 1767) Goodey, 1945, was at one time common in paperhanger's and bookbinder's pastes. Goodey, 1945, has described a very similar species, *P. redivivoides* which occurred in a banana maize-meal cider culture at Cambridge. The culture was used for rearing *Drosophila*. *P. ludwigii* (de Man, 1910) Goodey, 1945, has been recorded from the slime flux of certain trees, and also *P. pycnus*. This last habitat is probably the primitive habitat of the genus, all the others owing their existence to man's activities.

Whilst *P. silusiae* readily multiplies in suitable media, there is need for frequent dispersal from one habitat to another because of the impermanence of such media. The recorded association between insects, particularly *Drosophila*, and the habitats of species of *Panagrellus*, suggests that these flies may carry the worms from one locality to another. Several examples of nematodes being dispersed in this way are known. Aubertot (1923) found one of his potato puree cultures infested by *Rhabditis pellio* after being visited by *Drosophila*. He later found the larvae of *Rhabditis pellio* on the bodies of the flies and described how the larvae formed waving tufts on the surface of the medium, from which they attached themselves to anything with which they came in contact. Bovien (1937) has reviewed the associations between nematodes and insects. He quotes several examples of nematodes being conveyed from place to place by flies and beetles. Ensheathed

larvae of *Rhabditis coarctata* are recorded by Triffitt and Oldham (1927) as being transported from one patch of dung to another attached to the cuticle of *Aphodius fimetarius* and other species of dung beetles. Larvae of another species of *Rhabditis*, namely *R. curvicaudata*, actually coil themselves around the abdomen of Psychoda flies which visit sewage filter beds (Goodey, 1943).

One of the difficulties of accepting the view that *P. silusiae* and other members of the Turbatricinae are normally dispersed by insects, is the fact that they appear to lack the desiccation-resistant stage found in the examples quoted above. Aubertot (1923) has recorded for *P. silusiae* and Henneberg (1900) and Peters (1928) for the vinegar-eelworm, *Turbatrix aceti*, that the worms will not survive desiccation. Aubertot believed, however, that although the adult worms might perish whilst being carried by flies over anything but the shortest distances, larvae within the uterus could survive over longer periods. The author decided to investigate the resistance of *P. silusiae* to desiccation and to investigate other physiological characteristics which have bearing on its probable methods of dispersal.

Experimental work on desiccation resistance of *P. silusiae*.

Worms, in all stages of development, from active cultures in porridge were washed free from all traces of the medium and placed in water. Two drops of this new culture were spread uniformily over the depression in a cavity slide. Ten slides were prepared in this way and air dried under an electric light bulb at 18°C. It was difficult to decide exactly when the films were quite dry but the time when all movement of the worms had ceased was chosen to represent that condition. Each slide was left for ten minutes and then wetted, when it was found in all cases some of the nematodes recovered their activity. The experiments were repeated several times, extending the period of desiccation by five minutes each time. In this way it was found that the worms could survive desiccation for periods up to half an hour and resume normal activity after wetting. Recovery took up to one hour.

It was suspected that although recovery might not occur within an hour after longer periods of desiccation, ultimate recovery might be possible. To test this, slides with worms desiccated for three hours were wetted and kept wet for several days. Observations were made at frequent intervals and from a hundred slides thus treated, one observation was made three hours after the addition of water, of a single larva actively moving. This evidently represented a larva which had escaped desiccation due to protection within the body of the

mother. It agrees with Aubertot's conclusion but sets a limit of three hours on the maximum time of desiccation which protected larvae can survive.

It was further suspected from observations made on desiccation of porridge cultures that the presence of colloids in the medium " protects " the nematodes in some way from the ill effects of desiccation. It was decided, therefore, to place the worms in a colloidal solution, allow it to gelate and study the resistance of the nematodes to desiccation within the gel. To this end a culture of *P. silusiae*—reared in the nutrient medium previously described by the author, (Lees, 1950)—was mixed with a 10 per cent. agar solution at 35°C. The mixture soon set to a very stiff gel. This was cut into cubes of 8 ccm. which were placed in a desiccator for one week, at the end of which they were extremely hard and could only be smashed with difficulty by a hammer. The dry pieces were allowed to imbibe water for several hours ; the resultant jelly was broken up into small fragments and mixed with further water and the mixture examined under a 2-inch objective. Several specimens of *P. silusiae* were seen in active movement.

Specimens of the nematode in agar gel cubes have been similarly desiccated and kept in a dry condition for two months. On soaking the agar, they have been recovered in an active state. This embedding treatment may be likened to an artificial encystment. As encysted forms of animals are frequently more resistant to temperature extremes, it was decided to compare the range of temperature which *P. silusiae* can tolerate when in normal cultures with that tolerated when in an agar gel. The normal temperature range is from 1°C. to about 40°C. It was found that " embedded " worms could not withstand temperatures above 40°C., but could stand —5°C. for several hours and still lower temperatures for a few minutes, and could be recovered in an active state when the gel was wetted.

All specimens of *P. silusiae* recovering from any lengthy desiccation pass through a phase in which their movement is quite unlike that of the normal worm. The body is not thrown into the characteristic sigmoid curves but undergoes a peculiar vibratory movement, the hinder region oscillating from side to side. As recovery becomes more complete, the oscillations increase in amplitude and the body becomes curved. Ultimately the movements of the body permit the worm to move forward in the medium and free activity is regained. The cuticle of desiccated specimens of the worm may show considerable distortion and air bubbles may be visible in the tissues ; nevertheless recovery is possible in many cases.

Water may be removed from the body of an animal in several ways. One way is to subject the animal to desiccation by evaporation, as described for *P. silusiae* in the previous paragraphs. Another way is to place the animal in a hypertonic solution. To test the effect of removal of water in this latter manner, specimens of *P. silusiae* were placed in solutions of glucose of molar, two molar, three molar and four molar strengths. The solutions were placed in covered cavity dishes and about a hundred worms, in various stages of development, were added to each solution. Except in the four molar solution the worms sank to the bottom as they do in pure water. The four molar glucose solution had a sufficiently high density and viscosity to allow the worms to move freely at all depths. The worms in the molar solution of glucose remained active for several days, until the culture solution had become heavily infected with fungi and bacteria. Evidently the nematodes can withstand the external osmotic pressure of this solution, probably by emitting osmotically active substances, as found by Stephenson (1942, 1944) for *Rhabditis terrestris*. After three hours the worms in the two molar glucose solution showed reduced activity and many ceased moving after six or seven hours. Those in the other two solutions became quiescent more quickly. However only in the four molar solution were the worms completely motionless within one hour. Quiescence was accompanied by a marked change of shape in the worms; they took on a more stumpy form than the normal. In normal adult female *P. silusiae* the length/greatest breath ratio lies between 22 and 28 ; in the quiescent individuals the corresponding ratio fell between 15 and 20. Considerable distortion of the internal organs and of the cuticle was evident when these worms were examined microscopically.

Worms which had been immobilized for two days in four molar glucose solution were transferred to water. No immediate recovery was observed but after thirty minutes certain of the worms exhibited slight twitching movements characteristic of specimens recovering from desiccation. After three hours about 5 per cent. of the worms showed a considerable amount of movement, although none exhibited the type of progression characteristic of the normal animal. After three days the recoveries had risen from 5 per cent. to about 80 per cent. and normal active movement had been resumed. Larvae were more numerous than adults and in many cases active larvae were seen escaping from the dead remains of the mother.

The period of immobility in four molar glucose solution has been found to be capable of prolongation up to fourteen days. The worms had then sunk to the bottom showing that glucose must have entered

the body of the worm. Immobility appears, therefore, to be the result of an initial withdrawal of water from the tissues followed by a high concentration of glucose around them. Since in the three molar solution, there is some recovery of mobility after twelve hours among worms that were previously quiescent, the initial withdrawal of water is probably the more important factor in bringing about immobility.

A culture of inactive worms kept in four molar glucose for fourteen days was subjected to low temperature treatment at —5°C. for one hour. The solution did not freeze at this temperature because of its high solute concentration. After refrigeration, the culture was diluted and observed at frequent intervals. No sign of activity was visible during the first forty-eight hours following dilution but a few hours later certain of the worms were seen to be shaking slightly and a few larvae were observed escaping from the remains of the females. During the next two days larvae continued to escape in this way until they swarmed in the culture. None of the adults recovered. Larvae within the uterus are evidently more resistant to low temperatures when in a state of induced dormancy than are the adults.

Discussion on desiccation resistance of *P. silusiae*.

The experiments described show that if specimens of *Panagrellus silusiae* are rapidly dried, as occurs when water in which they are placed evaporates at room temperature, they are sensitive to desiccation and cannot endure a dry cuticle for more than a few minutes. If water is lost from their immediate environment at a slower rate, as occurs when the worms are embedded in a colloidal gel, they appear to be able to pass into a state of dormancy during which they can withstand desiccation over a considerable length of time. In this dormant state the resistance of adults (and to a greater degree the resistance of larvae within the uterus) to low temperature is increased. It appears that the tissues of the nematode can accommodate themselves to a considerable degree of dehydration, if time for the accommodation process is available. In the case of the colloidal gel experiments it might be argued that the gel prevented the tissues from ever becoming dehydrated ; the experiments using solutions of high osmotic pressure suggest, however, that dehydration of the tissues can be tolerated without death ensuing.

These observations on desiccation resistance in *P. silusiae* agree with those reported by different authorities on other nematodes. Even species which have gained a reputation for resistance to desiccation and low temperature can rarely withstand rapid drying. Menzel (1920) has reported that species of *Plectus*, *Dorylaimus* and other nematodes

inhabitating Spitsbergen mosses can withstand desiccation within the moss for two years ; rapid desiccation on a microscope slide is, however, fatal. Luyet and Hartnung (1941), after pointing out that in general the vinegar eelworm is poorly resistant to desiccation and freezing, show that this worm after being subjected to preliminary desiccation recovered after solidification in liquid air (—190°C.). The worms died within a few hours after recovery however.

These experiments on the desiccation resistance of *P. silusiae* suggest that the worm should be able to survive transport from one medium to another on the bodies of insects. They further suggest the possibility of the worms surviving in a dormant condition in dried up fragments of suitable media and these desiccated fragments serving to disseminate the worm. It was decided to test whether further physiological attributes of the worm favoured insect dispersal.

Observations on taxes in *P. silusiae*.

If *P. silusiae* is cultured on oatmeal porridge, the worms appear to abound on the surface but are less frequent within the medium. Peters (1928) states that the vinegar eelworm shows a similar preference for the surface and attributes it to a negative geotaxis. He suggests this taxis is independent of dissolved oxygen. In order to test the chemotactic response of *P. silusiae* towards oxygen, a small glass cylinder was taken and a glass partition fitted into it. The partition was cemented in place with glass cement and divided the cylinder into two halves, to within an inch of the bottom. Culture medium containing living worms was then placed in the bottom of the cylinder, so that the surface of the medium came just above the level of the bottom of the partition. Oxygen, led through rubber tubing and bubbled through water to remove some possible impurities, was allowed to blow gently through the orifice of a pipette on to the surface of the medium on one side of the partition. Nitrogen was similarly allowed to blow on to the other side. After an hour of this treatment, samples of equal volume were taken from each side of the partition. The samples from the side over which oxygen passed contained approximately twice as many worms as those from the other side. The experiment was repeated several times and in all cases the number of worms on the oxygen side of the partition greatly exceeded that on the nitrogen side. *P. silusiae* therefore appears to display a positive oxygen chemotaxis.

If a porridge culture containing *P. silusiae* is placed in glass tubes of one inch diameter and the tubes corked so that the air above the culture becomes saturated with water vapour, after twelve hours or

more worms may be seen moving up the glass sides. The worms can only climb if the medium is above a certain viscosity. In a strongly diluted medium, worms never leave the surface for the walls of the vessel. The most frequent migration upwards seems to commence in the dark, but in the daylight worms will move up the side farthest from the incident light. Rotation of the tubes causes the worms to move to the less strongly illuminated side; thus they show a negative phototaxis. Some worms reach the top of the tube after several hours, having ascended a distance of between three and four inches. Examination of the worms reveals that for the most part they are mature females ready to shed larvae. It appears therefore that whilst *P. silusiae* is attracted to the surface of the medium by a positive aerotaxis at all times, only the gravid females tend to climb above the surface in response to a negative geotaxis.

Observations on actual dispersal by insects.

Oatmeal porridge cultures of *P. silusiae* kept in a thermostatically controlled greenhouse at 60°F. soon attracted considerable numbers of the Vinegar Fly, *Drosophila funebris*. No flies of this species had frequented the greenhouse before using it to house eelworm cultures. In order to test whether the flies would act as dispersal agents for the worms, certain Petri dishes containing active cultures of *P. silusiae* were left uncovered. Other Petri dishes containing oatmeal porridge, to which a little malt vinegar had been added but free from nematodes, were placed in different parts of the greenhouse. The porridge surface was kept moist by the daily addition of water. The Vinegar Flies visited all the Petri dishes and spent a considerable time both walking and resting on the surface of the cultures. At the end of a week all the Petri dishes originally devoid of nematodes were found to contain numerous *P. silusiae*. Vinegar Flies were caught from time to time from the population resident in the greenhouse and after being chloroformed were examined for the nematode. Many worms were recovered in this manner from the legs of the flies. As previous experiments had led the author to expect, the worms most frequently obtained were gravid females. Muscid flies, which also frequented the greenhouse during the period of the experiment, were captured and examined for specimens of the worm. No specimens of *P. silusiae* were ever recovered from the Muscids nor did the latter visit the Petri dishes except on rare occasions.

Discussion on dispersal of *P. silusiae*.

Drosophila funebris has been shown to act as an effective dispersal agent for *P. silusiae* under conditions prevailing in a heated greenhouse. There seems to be no reason why the fly should not act in the same way " in the field." The observations of Aubertot (1925) suggest that Vinegar Flies can, in fact, carry *P. silusiae* greater distances than did the flies within the greenhouse, in the author's controlled experiments. The limit to which the worms can be carried would appear to depend upon the extent to which they can resist desiccation and the author has shown that under certain conditions this may be considerable. It is particularly significant that the gravid female exhibits a behaviour reaction likely to cause her to ascend the legs of an insect visiting the medium she inhabits ; as the gravid female, of all individuals of the species, is the most likely to colonize a new habitat successfully. Even if she suffers desiccation on the journey, the larvae within her uterus may live, and should she herself survive, her numerous progeny will rapidly form a new colony in a suitable medium.

The Turbatricinae have become in the course of their evolution one of the most thoroughly domesticated of the nematodes. Originally their habitats must have been places where plant tissue was undergoing acetic fermentation, for example rotten fruit and " slime-fluxes " of trees. Certain species for example *Panagrellus ludwigii*, *P. pycnus* and *Turbatrix aceti* var. *dryophila* appear to be confined to these habitats at the present day. *Drosophila* regularly visits material undergoing acetic fermentation and they would serve to disperse the nematodes. *Panagrellus silusiae* and *Turbatrix aceti* v. *aceti* could readily have been brought by this agent to their present habitats, which owe their existence to man's activity. Both worms appear to have become adapted to these man-made habitats to the extent that they are not found elsewhere. At the same time, they retain their original method of dispersal by *Drosophila*.

Summary.

1. *P. silusiae* is shown to be unable to resist desiccation on a microscope slide for periods above half an hour, although larvae within the uterus of the mother can survive for somewhat longer periods.

2. When " protected " by a colloidal gel, the worms are shown to resist desiccation for several months. Such " protected " worms are more resistant to temperature extremes than " non-protected " ones.

3. *P. silusiae* is immobilized in glucose solutions above 1M strength, although it can resume mobility on dilution of the solution, even after fourteen days in 4 M glucose solution.

4. *P. silusiae* is shown to be positively chemotactic towards oxygen, in addition the gravid females are negatively geotactic.

5. *Drosophila funebris* is shown to act as a dispersal agent for *P. silusiae*.

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Dates of Publication of Vol. XXVI.

Part 1 ... ... 18th March, 1952

Parts 2/3 ... ... 1st August, 1952

Part 4 ... ... 31st December, 1952

Corrigendum in Vol. XXVI, page 164.

$$\text{For } 100\times\frac{1+\frac{k}{m}-P_n{}^n}{1+\frac{k}{m}+P_n} \text{ read } 100\times\frac{1+\frac{k}{m}-Pn}{1+\frac{k}{m}+Pn}$$

Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, pp. 105—106.

Tom Goodey, 1885–1953.

Almost on the eve of his departure for Australia to advise the government there on nematological research, Tom Goodey, O.B.E., D.Sc., F.R.S., died suddenly on the 7th July, 1953, from a heart attack : he collapsed on his way home from a meeting of the Society of Friends.

Born at Wellingborough on the 28th July, 1885, Goodey was educated at the local Board School, at the County School, Northampton, and at the University of Birmingham whence he graduated with Honours in zoology and botany in 1908. After some sound early work on marine zoology (4 papers) he became a protozoologist, working at Rothamsted (1912–13) on the role of soil protozoa in the phenomena of partial soil sterilization, and at Birmingham, in World War I, on amoebic dysentery (9 protozoological papers). After one year (1920) as Plant Helminthologist at Rothamsted, he joined Leiper's staff at the London School of Tropical Medicine and later at the Institute of Agricultural Parasitology, where he was the senior member of the staff throughout the duration of the Institute. When the latter ceased to exist in 1947, on Leiper's retirement, its plant-nematode half arose, phoenix-like, as the Nematology Department at Rothamsted with Goodey as Head of Department. Although he retired from this office in 1952, he continued to work full time in the department where, freed from administrative worries, he was actively engaged at the time of his death in preparing (with the assistance of J. B. Goodey) a new edition of his first text-book, " Plant Parasitic Nematodes ", and in working on the genus *Anguina*.

From 1920 he worked exclusively with nematodes, publishing about 100 papers of which nearly 90 per cent. appeared in this Journal. He has some 19 papers on the nematodes of mammals but, when he joined the new Institute in 1924, he soon specialized in the plant-parasitic and free living species now grouped under the name " Nematology ", and it is mainly in this field that he soon became acknowledged as a world authority.

Volume I of this Journal (1923) opens with a joint paper by Goodey and Cameron, and his name appears in every volume down to 1949 with an average of over three papers a year. The fall to three papers in

five years after 1948 merely reflects his absorption in administering his Department. Most of these papers reveal the patient and meticulously careful work of the taxonomist which Goodey pre-eminently was, but he was never merely that; in 1930 the *Phil. Trans.* paper on the life-history and development of *Tylenchinema oscinellae* in *Oscinella frit* (summarized in Vol. VIII of this Journal) well shows the range of his biological interest. In Vol. X (1932) came his long, masterly survey of the genus *Anguillulina*, and in the following year his first text-book was published. In 1940 his valuable compilation on "Nematode parasites of plants catalogued under their hosts" was issued by the (then) Imperial Bureau of Agricultural Parasitology. During the next five years he interested himself in the stem-eelworm disease of onions and showed that infestation was carried by the seed, which could be disinfested by fumigation with methyl bromide. His last major work was the second text-book, "Soil and freshwater nematodes" (1951).

Apart from professional interests, Goodey had a fine tenor voice and was an amateur singer in a variety of media from folk songs to German Lieder and Oratorio. He will long be remembered for singing Midir in Boughton's "Immortal Hour" in the West End. He also had a keen interest in rock- and water-gardening.

He was long and actively associated with the Society of Friends, and had been for many years an Elder of the Harpenden Meeting. He leaves a widow, a son, and three daughters. Nematology is very much the poorer for his going, though he might well have approved of the sudden departure before old age had quenched his enthusiasm or dimmed his vision. His many friends sadly miss a man of great integrity, energy, humility and patience whose character was saved from the implied severity by an unrestrainable sense of fun.

B.G.P.

Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, pp. 107—112.

Vertical Migration of Potato Root Eelworm.

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Cysts of *Heterodera* species have been recovered from considerable depths in soil. Thus, Thorne (1939) found cysts of *H. schachtii* 24 inches below the surface, the lowest depth he investigated. The contents of cysts in the surface layer may be killed by insolation during a fallow. Soil fumigants may kill a proportion of eelworms in the upper foot of soil, but often are ineffective in the surface inch or two and at great depths. Deep ploughing may bury cysts. In all these cases it is important to know to what extent, and how quickly, disinfested layers of soil are recolonized, from either below or above, by larvae hatching from the cysts and migrating vertically.

The vertical migration of potato root eelworm larvae has been experimentally investigated by growing a potato plant in a wooden box of soil which was free from cysts apart from those artificially introduced in a thin layer at a known depth. At the end of the season, newly-formed cysts at various horizontal layers in the box gave quantitative evidence of the extent of migration.

Boxes were made by superimposing a number of sections each 2 inches deep. 48 of these sections were made from planed $2'' \times \frac{1}{2}''$ wood strips, each section measuring in plan 6″ square internally (7″ externally), and in elevation $7'' \times 2''$. The four sides of a section were nailed together and the section was secured by a single turn of stout galvanised wire externally. All sections were treated with Cuprinol, and that forming the base of a box was covered with perforated sheet zinc. For the two experiments described here the 48 sections were made into 8 boxes of 6 layers each ; thus each box would hold $6'' \times 6'' \times 12'' = 432$ cu. ins. Leaving the top inch clear of soil, for water, each box held about 6·5 litres (1·4 gal.) of soil. The 6 sections of a box were held together by passing a strip of 1″ aluminium vertically between the sides of the sections and the securing wires, bending the strip back over the top and bottom wires, on each of the four sides of the box. Prepared in this way the box was quite rigid, especially when full of soil.

At the end of the growing season each box was dismantled as follows. It was stood in a large enamelled tray and a pair of the aluminium strips was removed, the other pair being straightened at the top. A sharp knife passed between the 1st and 2nd sections severed roots and tubers, and the top section was lifted off, the exposed soil being pushed off into the tray with a straight-edge. Particles of soil lodged on the wires or strips were dusted off, the box removed, and the contents of the tray bagged for air-drying. This process was repeated for each section of each box, the 48 soil samples (each from a known depth) being subsequently treated in the usual way for recovery of eelworm cysts.

I. Inoculation Level *versus* Watering Method.

In the first experiment it was desired to compare downward migration from an inoculation near the top (T) with upward migration from one near the bottom (B). It was not clear whether hatched larvae would migrate actively or would be carried passively by percolating water, so half the boxes were watered from above (*t*) and half from below (*b*). There were thus duplicate boxes receiving each of the four treatments T*t*, T*b*, B*t* and B*b*.

Some 60 l. of fibrous loam, sifted through a sieve of 3 mm. mesh, was found to contain a few lemon-shaped *Heterodera* cysts. To be on the safe side, it was partially air-dried (to 25% water content) and fumigated in a cylindrical bin of 18″ diameter by injecting 5 ml. of undiluted ethylene dibromide 25 cm. deep and another 5 ml. 10 cm. deep. The bin's lid was forced on over a sealing sheet of tarred paper and the bin left for 4 days, after which the soil was repeatedly raked in a thin layer on a concrete floor in bright sun to disperse the fumigant. A potato grown in a pot of this soil failed to reveal any *Heterodera* cysts.

Soil was pressed down in the four T boxes to within 4½″ of the top, a tuber planted, more soil pressed down to within 1″ of the top, about 1,000 *H. rostochiensis* cysts sprinkled over the surface, and another ½″ of soil added. In the four B boxes the soil was pressed down to the top of the lowest section and the cysts sprinkled at that level. The cysts had been recovered from a heavily infested soil by flotation in Fenwick's apparatus, the float being concentrated by rolling until there were about 10 cysts per mg. of dry float; they were measured by volume in a small container holding about 100 mg. Detailed counts on four volumes gave a mean of 1,113 cysts with a standard deviation of 18·5%.

The 8 boxes were kept in a glasshouse to prevent any top watering of the *b* series, which were stood in a galvanized tray to which water was added periodically. The four *t* boxes were stood on an inverted tray (since *H. rostochiensis* was present in the soil of the glasshouse) and watered from above. The experiment was set up on the 6/5/49; potato shoots had broken soil by the 23/5 (the *b* series first); haulms were dying down by the 26/8 when they were cut off, watering ceased, and the boxes were left to dry out. It was not found practicable to give equal quantities of water to the *b* and *t* series: the soil surface of the former remained dry for a week after starting, and it was felt necessary to keep water in the tray continuously until the soil surface became moist.

On dismantling the boxes, tubers were found mostly in the 2nd and 3rd layers in the *t* series, but higher in the *b* series. Air-dried soil samples showed that each section except the topmost held from 1 to 1·4 kg. of soil. After thorough mixing 200 g. was weighed from each sample and the contained cysts floated out and counted: these counts, given in Table I, are less misleading than "total cysts per section" since the top section was only about half full.

A statistical analysis of all the data has been carried out, using a logarithmic transformation of the counts, but the results are not given here. A theoretical difficulty arises from the fact (for pointing out which I am indebted to M. J. R. Healy) that the six levels of a box cannot be randomized for position, from the nature of the case. Moreover, the essence of the experiments is to demonstrate the presence of eelworms in levels from which they were formerly absent, and for this purpose analysis is not especially useful. The data speak for themselves.

By washing the soil remaining in those samples which contained cysts, where necessary, sufficient cysts were recovered to set up a hatching test. Lots of 100 cysts were exposed to the action of potato root diffusate in an incubator at 24°C, the hatched larvae being removed and fresh diffusate added weekly until hatching had practically ceased. From counts of hatched larvae in 1 ml. aliquots the density of larvae per cyst was estimated and thence, from the cyst densities, larvae per gramme of air-dried soil. The two criteria (densities of cysts and larvae) led to identical conclusions, even in detailed comparisons of levels per treatment, and it would therefore appear that, in experiments of this type, little is gained by resorting to larval counts. Hatching in root diffusate was used instead of direct counts of eggs

and larvae from dissected cysts to ensure that no confusion arose from the original light infestation of *Heterodera* cysts in the fibrous loam.

Table I shows that there has been vertical migration of *Heterodera* larvae, both downwards and upwards, over distances of about 6 to 8 inches. The majority of cysts are found in the level in which the inoculum was placed or in the adjacent level. In the T*t* series there is a rise in cyst density from the top to the second level; this can be ascribed to a washing down of larvae, and probably of some of the inoculated cysts, by percolating water, since there is no sign of it in the T*b* series. A comparison of T*t* with B*t*, in the densities of Table I

TABLE I.

Final cyst density (cysts/200 g.) by levels: 1949.

Inoculation:		Top				Bottom			
Watering:		Top		Bottom		Top		Bottom	
Box No.		1	2	3	4	5	6	7	8
Level	1	81	98	107	179	0	0	0	0
	2	586	680	70	137	0	27	0	0
	3	186	298	20	87	63	144	0	0
	4	83	83	0	117	168	360	0	0
	5	13	0	0	0	496	496	0	0
	6	0	0	0	0	1413	494	155	137

TABLE II.

Total numbers of cysts by pairs of boxes: 1949.

Inoculation:	T Top	B Bottom	Watering Totals
Watering:			
t Top	12,134	21,133	33,267
b Bottom	3,500	1,561	5,061
Inoculation Totals:	15,634	22,694	38,328
Total cysts originally added:			8,000

and the estimated totals of Table II, suggests that more larvae form new cysts and migrate further from a bottom inoculation and against the stream of percolating water, than from a top inoculation with the stream, though the difference in densities is slight. Indeed, there was a 10-fold multiplication in B*t* compared with 6-fold in T*t*. Bottom watering as applied in this case, appears to be unfavourable even with top inoculation.

II. Inoculation Level *versus* Location.

The first experiment was carried out under glass in order that the " top watering " treatment might be fully controlled. Fenwick (1951) has shown that, when experimental 6-in. pots of potatoes were exposed to infestation in four locations, eelworm development was least under glass and that temperatures above 21°C. became increasingly unfavourable to the development of new cysts. In 1950 the vertical migration boxes were set up exactly as before but the comparison between top and bottom watering was replaced by a comparison

Table III.

Final cyst density (cysts/200 g.) by levels : 1950.

Inoculation :		Top				Bottom			
Situation :		Shade		Glass		Shade		Glass	
Box No.		1	2	3	4	5	6	7	8
Level	1	590	1106	257	31	1	7	4	6
	2	1042	1108	158	273	11	7	7	9
	3	263	224	18	46	17	10	5	7
	4	43	98	10	8	45	171	6	108
	5	3	7	3	1	379	701	661	1389
	6	4	2	5	1	527	974	2253	1627

Table IV.

Total numbers of cysts by pairs of boxes : 1950.

	T	B	Situation
Inoculation :	Top	Bottom	Totals
Situation :			
s Shade :	21,980	10,893	32,873
g Glass :	3,813	20,284	24,097
Inoculation Totals :	25,793	31,177	56,970
Total cysts originally added :			6,500

between the two locations : (*s*) outdoors on the north side of a building, and therefore in shade most of the day and (*g*) in a glasshouse as before. Top watering alone was used, the *s* series being watered whenever natural rainfall was inadequate. Inoculations, at both levels, were at the rate of about 800 cysts per box. In view of the previous results with larval counts, only cysts were counted.

The densities of cysts per 200 g. of air-dried soil are shown in Table III. The cyst densities of the T*g* boxes are much lower than in the other three treatments, as can be seen also from the cyst totals of Table IV, but the differences between the two inoculation totals (B-T)

and the two location totals (*s-g*) are slight : in each case the multiplication of cysts has varied from 8- to 10-fold. It is clear that the top-inoculation boxes have suffered severely under glass, where the original cysts were protected by only a half-inch cover of soil, and this is in line with Fenwick's findings in 6-in. pots. But Table IV shows that there is a considerable multiplication of cysts from bottom-inoculation under glass, the total number being comparable with top-inoculation in shade, and about twice as great as from bottom-inoculation in shade. It seems, therefore, that the eelworm flourishes under glass provided that it is protected from the direct effects of insolation. This fact would account for the considerable multiplication of *H. rostochiensis* in the soil beds of tomato glasshouses.

Summary.

A simple apparatus is described in which can be measured the vertical migration of *Heterodera rostochiensis* larvae in soil and in the presence of the host plant. The migrations upwards and downwards are comparable in extent, but can be inhibited by water-logged soil and greatly reduced if the cysts are exposed to insolation under glass when near the soil surface. In the layer furthermost from the inoculum (8 to 10 inches away), either no cysts are formed or their number constitutes a very small proportion of the total : a fraction of one per cent.

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Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, pp. 113—118.

Changes in Potato Root Eelworm Population with Time and Depth.

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Under the influence of a growing potato crop, changes occur in the potato root eelworm population of the soil during the course of a season. It is customary in this department to estimate the eelworm population in two stages: first the density of cysts per unit weight of soil is estimated and then the density of larvae (or unhatched eggs and larvae) per cyst. The product of these two gives the density of larvae per unit weight of soil, which is considered a fundamental estimate of eelworm population.

During a potato season the cyst density, measured in terms of cysts per gramme of air-dried soil and symbolized c/g, will remain constant for some weeks and then begin to rise as new cysts are formed on the roots of the crop. The estimate of larvae per cyst (l/c), since it necessarily excludes larvae which are migrating through the soil or have already penetrated the roots, will at first fall owing to hatching and will not rise until the new generation of cysts appears. The third estimate of larvae per gramme of soil (l/g) will thus be misleadingly low until near the end of the season when it will again become a reliable estimate of eelworm population.

Methods.

It was desired to investigate these changes by measuring cyst and larval densities at different times during the season and also at different depths in the soil. For this purpose potatoes were grown in infested soil in boxes made up of sections 2 in. deep. The construction and use of these sectional boxes have been described elsewhere (Peters, 1953). The available 48 sections were made up into 8 boxes, each 6 sections (12 in.) deep. The bottom section contained mostly drainage material (and was not sampled); the rest of each box was filled with a naturally infested allotment soil and a tuber was planted on the 17th April, 1951. The 8 boxes were stood outside to the north of a building so that they were protected from insolation most of the day.

One pair of boxes was brought in on each of 4 dates, respectively 5, 9, 13 and 19 weeks after planting; the haulms were cut off and as soon as the soil was dry enough for handling the pair of boxes was dismantled, all the soil from each of the top 5 sections being separately

bagged and air-dried.

At the end of the experiment, two samples of 200 g. of air-dried soil from each section were washed for cyst counts, the remaining soil also being washed to provide additional cysts. From each section two batches of 100 cysts each were subjected to the hypochlorite technique for counting eggs and larvae : the 100 cysts were bisected by Hagedorn needle and washed in a (nominal) 1% solution of calcium hypochlorite for 30 minutes, the solution then being diluted to 100 ml. and eggs and larvae counted in two 1 ml. aliquots in an eelworm counting slide.

Analysis.

Counts were analysed in logarithmic transformation, taking log $(l/g) = \log (c/g) + \log (l/c)$. The analysis yields various treatment means the antilogs of which are actually geometric means ; these are considered more appropriate than arithmetic means to population studies of this kind, and are the values listed in Table 1. The table gives, for each of the criteria (c/g), (l/c), and (l/g), the geometric mean values for each level at each time, together with the general mean for that time. The symbol d placed between any two values, horizontally or vertically, means that the two values differ significantly.

The direct arithmetic means of these criteria tend to be correlated with their standard deviations, thus rendering them unsuited to the " analysis of variance " procedure. This difficulty is largely obviated by using the logarithmic transformations, the analyses of variance and subsequent tests of significance being carried through in the units : log $(c/200g)$, log (l/c), and log (l/g). The use of log $(c/200g)$ instead of log (c/g) (which would entail troublesome negative logs) does not affect the analysis of variance since it merely entails an added constant.

Table II gives analyses of variance for the three criteria. In each case line (a) measures the dispersion of the Time means (over all levels) about the general mean. The appropriate error variance for testing its significance is in line (b), measuring the variation of each pair of boxes (plants) about their own mean. Significance attains a very high level, and Table I shows that the only non-significant change in the Time means is that between cyst counts at times I and II. The exceptionally low values in lines (b), showing low variability between the plants of each pair, are not easily explained. It would be reasonable for this mean square to be larger than that of line (e), and for this reason the latter has been used as error variance in comparing the Time means instead of line (b).

Line (c) measures the dispersion of Level means (over all times) about the general mean. These means are of no special interest in the present situation where, as line (d) shows, there is significant interaction between Times and Levels. Interest centres in the comparisons of the five levels at any one time (the vertical columns of Table I) or the four times at any one level (the horizontal lines of Table I). The appropriate error variances for these two kinds of comparison require some discussion.

TABLE I.

Geometric mean counts of cysts per g., eggs and larvae per cyst, and eggs and larvae per g. of soil.

Criterion	Level	Time : I		II		III		IV
	1	·516		·536		·519	*d*	2·931
						d		*d*
	2	·470		·610	*d*	1·439	*d*	10·739
c/g	3	·507		·526	*d*	1·774	*d*	9·550
						d		
	4	·480		·539	*d*	·927	*d*	7·550
	5	·464		·607		·659	*d*	6·934
	Mean	**·487**		**·562**	*d*	**·959**	*d*	**6·912**
	1	53·0		44·8	*d*	17·9	*d*	243
				d		*d*		
	2	37·8	*d*	12·8	*d*	73·4	*d*	210
l/c	3	45·8	*d*	15·8	*d*	78·7	*d*	211
						d		
	4	60·4	*d*	11·2	*d*	45·2	*d*	261
	5	62·5	*d*	17·2	*d*	29·2	*d*	223
	Mean	**51·1**	*d*	**17·7**	*d*	**42·4**	*d*	**229**
	1	27·4		24·0	*d*	9·29	*d*	714
				d		*d*		*d*
	2	17·8	*d*	7·82	*d*	106	*d*	2254
l/g	3	23·2	*d*	8·30	*d*	140	*d*	2014
						d		
	4	29·0	*d*	6·05	*d*	41·9	*d*	1972
	5	29·1	*d*	10·4		19·3	*d*	1549
	Mean	**24·9**	*d*	**9·96**	*d*	**40·6**	*d*	**1582**

For the vertical comparisons the error variance of line (e) has been used : this represents the interaction between the two plants and five levels at any one time, receiving 4 degrees of freedom from each of the four times. I am indebted to M. J. R. Healy for pointing out that this is not completely sound, since the five levels for any one plant are not independent ; the eelworm data are correlated with the behaviour of successive levels of a single root system. It is akin to treating successive measurements on a single animal, at different times, as if they were independent. Put otherwise, the different times of the latter

case can no more be randomized than the five levels of the present case. Nevertheless, the difficulty having been noted, and no marked trend in the data having been observed, the standard procedure has been followed.

TABLE II.

Analyses of Variance.

	Degrees of Freedom	Mean Squares for 3 Criteria : log ($c/200g$)	log (l/c)	log (l/g)
(a) Times (T)	3	5·656410***	4·262581***	18·668536***
(b) Plants/Time	4	·007888	·024072	·034169
(c) Levels (L)	4	·182522	·009860	·190798
(d) Interaction TL	12	·073151**	·183221***	·397018***
(e) P/T × L/T	16	·017920***	·016992	·052552*
(f) Duplicates	40	·003309	·016047	·023569
Total	79	·240858	·202985	·803206

Asterisks indicate significance at the 5%*, 1%**, and 0·1%*** levels.

I am also indebted to Healy for explaining that the horizontal comparisons of Table I should theoretically be dealt with by the split-plot technique. Taylor (1950) gives details of a method whereby an error variance can be compounded from lines (b) and (e), with a formula for finding the appropriate degrees of freedom. This would be applicable if error (b) were larger than error (e) ; in fact it is smaller in this case for log ($c/200g$) and log (l/g), and therefore line (e) has again been used for computing error variances. For log (l/c), where (b) is larger than (e), Taylor's method has been applied : it gives a slightly more stringent test but does not affect the particular differences deemed significant by merely using error (e).

Line (f) measures the basic dispersion of duplicate cyst counts and of the two batches of 100 cysts used for counting larvae ; its sole purpose is to show that laboratory technique has been up to standard.

In Table I those differences between vertically or horizontally adjacent values have been taken as significant when they have exceed t times their standard error, computed from line (e) of the analyses in Table II, and using the 5% significance level at which, for 16 degrees of freedom, $t = 2{\cdot}120$.

These critical differences, 2·12 (S.E.), apply to the comparison of means of logarithmic values : their antilogs therefore represent critical ratios applicable to the geometric means of Table I. The values of these ratios for the body of the table are : (c/g) 1·588 ; (l/c) 1·568 ;

(l/g) 2·206. This experiment is therefore sensitive to population changes of 59% in cyst density, 57% in eggs and larvae per cyst, and 121% in eggs and larvae per g. of soil, in the comparison of pairs of sections. The corresponding values for the general Time means are 23%, 22%, and 42% respectively.

Results.

Time Means.

The cyst density shows no significant change between the 5th and 9th weeks, though the 15% increase may well represent a real effect. The formation of new cysts leads to a significant increase at the 13th week and to a marked further increase at the 19th. Over the whole period there has been a 14-fold increase in cyst density, though the increase at the 13th week was only 2-fold. The major increase in cyst density occurred in the last 6 weeks (late July and August).

Table I gives no indication of what larval densities were at the start of the experiment. A sample of the infested soil taken at that time showed 71·938 l/c. Thus, by the 5th week there is already an appreciable fall, due to larvae hatching and leaving the cysts, with a further significant fall by the 9th week to 25% of the starting value. The 13th week (Time 3) shows a significant rise, due to the formation of new cysts containing eggs, but it is still only 59% of the starting value. As in the cyst counts, it is the last 6 weeks which show the most marked change in eggs and larvae per cyst, a 5·4-fold increase in the last period leading to a final 229 l/c, nearly 3·2 times the original density.

The experiment started at 35·033 eggs and larvae per gramme of soil. Thereafter this density-index roughly follows l/c except that by the 13th week it has just passed the starting value. This suggests that, after 3 months' growth of a crop the eelworm population would show little net change. But in the present case the potatoes were growing under very artificial conditions, in boxes and in the shade, and the hypochlorite technique does not measure the viability of eggs and larvae : the 41 l/g at Time 3 may represent a much higher viable population than the 35 l/g at the start. The increases in c/g and l/c during the final 6 weeks lead to a 39-fold increase in l/g during that period, or 45-fold over the whole experiment.

Making all allowances for the artificialities of the experiment, this result at least suggests that the rapid increases in eelworm population observed in the field are largely due to changes which occur late in the season, and would be less likely to be found with Early varieties grown for a shorter time.

Level Means.

The data in the body of Table I necessarily follow broadly the course discussed above; of interest here are those levels which significantly depart from this average course.

The first point to notice is that there are no significant differences between levels at Time I, in any criterion. The same is true of c/g at Time II, though the significant fall in l/c at this time shows the top level lagging behind the rest. By Time III, levels 2, 3 and 4 show a significant increase in c/g, levels 2 and 3 being significantly higher than the rest. Presumably roots would be fewer, and later in arriving, in level 5, some 8 inches down (when the soil in the boxes had finally settled, there was only 1 inch in level 1). The criterion l/c at Time III shows signicant increases at levels 2 to 5, but a significant *decrease* at level 1: this is further evidence that the natural changes proceed more slowly in the top level. At this time (13th week) the picture is of level 3 leading, closely followed by level 2, but with a significant lag upwards (1) and downwards (4 and 5): this is slightly more evident in the criterion l/g.

Time IV shows significant increases at all levels in all criteria, compared with Time III. At this time there are no longer any significant differences between levels in l/c, and in c/g the only significant difference is that level 1 is lower than all the others. This difference is reflected also in l/g. After 19 weeks there are fewer cysts and (eggs + larvae) per g. of soil in the top inch than below, though even here there has been a 5·7-fold increase in the cyst density and a 20-fold increase in the density of eggs and larvae, per unit weight of soil, compared with the start of the experiment.

Summary.

By growing potatoes in infested soil in sectional wooden boxes, changes in the population of potato root eelworm have been observed at 5, 9, 13 and 19 weeks after planting, and at 5 two-inch levels in the soil. Results show that most of the increase in population occurred during the last 6 weeks of the experiment and that the normal population changes proceeded more slowly in the topmost level, leading to a lower final population in that level.

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Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, pp. 119—128.

Population Studies on the Potato Root Eelworm (*Heterodera rostochiensis* Woll.)

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Although many experiments have been conducted on the chemical control of *H. rostochiensis* results generally have been rather disappointing. The more commonly advocated method for controlling the parasite is the practice of suitable crop rotations in which potatoes are grown only infrequently : as there is little information available on eelworm population changes in the presence and absence of potatoes the nature of a suitable rotation is largely a matter of opinion. Peters (1949) observed that when potatoes are grown in the presence of a light infestation of potato-root eelworm, the latter multiply by a " compound interest law " the factor for which varies with climatic and soil conditions. In the absence of potatoes the eelworm population decreases due to natural mortality and spontaneous hatching ; the latter factor probably amounts to about 50 per cent. per year (Fenwick, 1950, Oostenbrink, 1952). The present paper presents some data on population changes in the presence of potatoes.

In all experiments herein described unless otherwise stated, potatoes were grown in 6-in. pots of naturally infested stiff loam from experimental plots at Rothamsted, varying levels of infestation being achieved by admixture with a similar soil carrying a negligible infestation. In a few cases eelworm-free loam was mixed with sand in the ratio 3 : 1 and cysts added. All soils were coned and quartered three times, all tubers were chitted before planting and all experimental pots were sunk in a gravel plunge.

When potatoes are growing, a substance is produced which stimulates larvae to hatch from any cysts near the roots, so one would expect the primary effect of growing potatoes on infested land to be a decrease in the number of larvae per cyst. To estimate the magnitude of this decrease, chitted tubers of the variety " Arran Banner " were planted in infested soil, the plants being lifted at different times. Replication was six-fold. The soil left after removing the plants was dried, the cysts recovered and estimations made of their larval content by dissection (Fenwick, 1952). The potatoes were planted in May, 1949 in two types of naturally infested soil, one from Rothamsted, the other

a black fen soil from the Chatteris area of Cambridgeshire. They contained 1.7 and 1.8 cysts/gm. respectively with a larval content of 159 and 61.8 larvae per cyst corresponding to 272 and 112 larvae per gm. of soil. The first examinations were made 4 days after the potatoes broke surface, 12 days after planting : cyst counts indicated that no new generation of cysts had been liberated into the soil before the 40th day after planting. The larval contents of cysts from both soil types for different times after planting are given in Table I.

TABLE I.

Showing fall in larval content of cysts in the presence of potato plants : data given as larvae/cyst.

	Initial content.	Time in days after planting.				
		16	19	26	33	41
Rothamsted soil	159	66	76	40	20	34
Chatteris soil	61.8	46	42	23	6.9	11

It is clear that the growing potatoes effected a decrease in the larval content of the cysts. Rothamsted cysts fell from 159 to 20 larvae per cyst in 33 days while Chatteris cysts fell from 62 to 6.9 larvae per cyst in the same time, corresponding to 87.5 and 89 per cent. larval emergence respectively. At 41 days a rise in larval content occurred, presumably due to the formation of new cysts, thus it seems that in the presence of growing potatoes the degree of emergence from cysts in the vicinity of the plant is about 80—90 per cent. up to the time of white cyst production. Since root diffusate production probably continues as long as the plant is growing it seems reasonable to suppose that so also does larval emergence, so that by the end of the growing season the old cysts are probably to all intents and purposes emptied and the infestation present in the vicinity of roots towards the end of the season is due almost entirely to the new generation of cysts formed in the roots.

A different result was obtained from a similar experiment using Rothamsted soil in 12-in. pots. The initial larval content of the cysts used was higher in this case being 90 larvae per cyst, falling to 49 larvae per cyst in 49 days—a larval emergence of 45.6 per cent. It was considered that two factors might account for this discrepancy. The pots used in this experiment were not sunk in gravel plunges as it was considered unnecessary for so large a pot and is possible that during hot summer weather, the soil temperature may have risen sufficiently to partially inhibit hatching ; also the root systems at the end of 49 days

had not penetrated the whole soil mass and possibly many cysts round the periphery of the pots had not been influenced by the active diffusate. To investigate this more thoroughly a third experiment was set up in 1951 : chitted tubers of the variety Arran Banner were grown in 12-in., 8-in. and 5-in. pots of soil containing 1.2 cysts per gm. with an initial larval content of 94 larvae per cyst—corresponding to 113 larvae per gm. of soil. Replication was three-fold. Plants were lifted at break-through (14 days after planting) and at 37, 44 and 56 days after planting. The soil left behind was washed, the cysts recovered and dissected.

TABLE II.

Larval contents of cysts in different sized pots at different times after planting potatoes.

Time from planting.	Size of pot.			mean.
	12 in.	8 in.	4 in.	
14 days	41	67	71	62
37 days	28	28	17	20
44 days	20	16	21	19
56 days	28	23	23	28
Mean	31	33	36	33

The larval contents are given in Table II. Cysts recovered from a parallel series of pots without potatoes showed no significant decrease in larvae per cyst with time. Analysis of these data shows that pot size had no significant effect on larval emergence but the increasing degree of larval emergence up to 44 days is confirmed. The tentative conclusion was drawn that up to a distance of 6 in. from the root centre the effect of diffusate produced by the plant is very great and probably at least 70 per cent. of the larvae leave the cysts in the first few weeks of growth. It is therefore reasonable to assume that in the vicinity of the root mass at the end of the season the soil infestation is mainly due to a new generation of cysts.

To investigate other changes in population, following the initial phase of cyst emptying, some of the original potatoes planted in Rothamsted and Chatteris soil were allowed to grow on to completion and the cyst and larval population periodically estimated. In Rothamsted soil the population rose to 6.05 cysts per gm. with a mean larval content of 80 larvae per cyst and a general level of 486 per gm. of soil. Compared with the initial infestation this corresponds to 356 per cent. increase in cyst number, a decrease of 48 per cent. in larvae per cyst and an increase of 184 per cent. in larvae per gm. of soil. The Chatteris

figures were 7.6 cysts per gm. of soil, 103 larvae per cyst and 760 larvae per gm. of soil—increases of 423 per cent., 167 per cent. and 708 per cent. respectively over the original infestation. The results are set out graphically in Figs. 1–3. It is interesting to observe that although the Rothamsted soil was twice as heavily infested at the start of the experiment as was the Chatteris soil, the latter was 48 per cent. more heavily infested at the end than was the former.

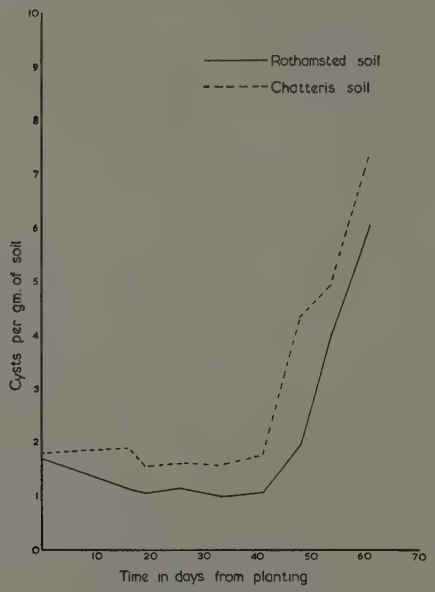

Fig. 1. Changes in cyst population in 6-in. pots in the presence of growing potato plants.

Concurrently with the above experiments investigations were conducted to ascertain the degree of larval penetration occurring during the early stages of growth up to the white cyst stage. To this end plants removed from soil for investigations into larval emergence, were carefully washed in running water till all soil adhering to the roots was removed and were then planted in 6-in. pots of eelworm free loam and sand and allowed to grow on to maturity. When they died back the

soil was dried and washed. Since cysts recovered were all derived from larvae already in the roots at the time of transplanting, it was hoped that the number of cysts recovered would serve as an index of root infestation at the time of transplanting. It was found however, that early transplants yielded more cysts than did later ones, the yield of cysts for potatoes transplanted at different times after breakthrough being as follows for the two soil types :—

Time in days	0	3	7	14	22
Rothamsted ...	7,685	4,361	6,990	3,185	211
Chatteris ...	2,227	3,687	3,697	1,792	89

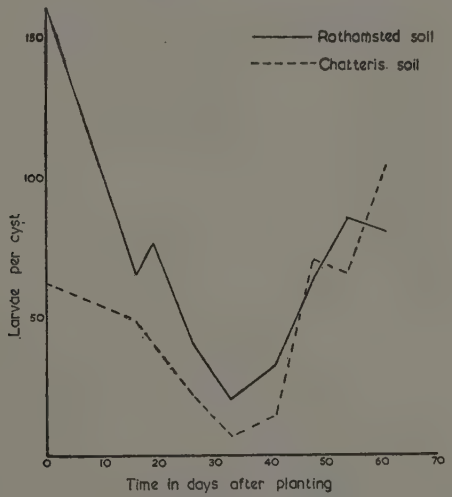

Fig. 2. Changes in larval content of cysts in 6-in. pots in the presence of growing potatoes.

The decline in cyst production following late transplanting is considered by the authors to be due to root damage incurred in the transplanting process. The root systems of the plants at breakthrough were small and could be easily handled but the rapid growth after breakthrough resulted in a large branching root which was extremely difficult to handle without damage. The above results can therefore only be considered of value as indicating the very high degree of larval penetration occurring before the potatoes break surface : in the case of Rothamsted soil an average of 7,685 cysts were recovered from potatoes

transplanted at breakthrough. Assuming a sex ratio of unity there must have been present an average of at least 15,000 larvae per root and since the weight of root at breakthrough was about 5 gms. the larval density must have been approximately 3,000 larvae per gm. of root. It is therefore not surprising that a potato crop growing on heavily infested land shows the characteristic symptoms of eelworm attack very soon. There was an interesting difference in cyst yields in this experiment from the two soil types—plants grown in Rothamsted soil yielded 7,685 cysts, those grown in Chatteris soil yielded 2,227, a ratio of 3.45 : 1 compared with a ratio of 2.43 : 1 for initial levels of soil infestation. Yet

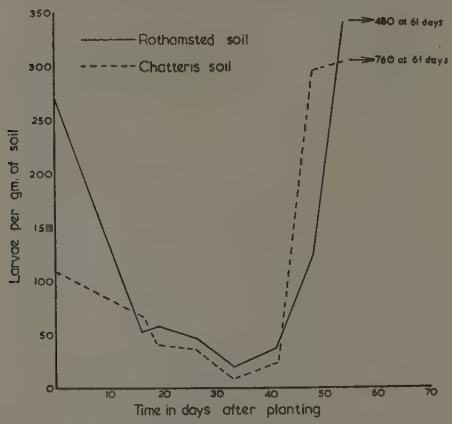

Fig. 3. Changes in numbers of larvae per gm. of soil in 6-in. pots in the presence of growing potatoes.

despite these differences in initial root infestations, plants grown undisturbed in Chatteris soil yielded 1.23 times as many cysts as did similar plants grown in Rothamsted soil. This phenomenon has been observed by the authors in other experiments and suggests that multiplication of eelworm in the field is not a matter amenable to simple arithmetic but is influenced by an interaction between host plant and parasite. It seems reasonable to assume that the mass invasion of roots in Rothamsted soil caused considerable disorganisation of the root tissues adversely affecting their growth and rendering them less suitable for the development of larvae which might invade later. The lighter

initial infestation of plants grown in Chatteris soil had less effect on the root system, and larvae invading at a later date would encounter more favourable conditions and finally yield a greater number of cysts than those in Rothamsted soil.

To investigate more precisely the effect of pot size in conjunction with the above effects an experiment was set up in which potatoes of the variety Arran Banner were grown in infested Rothamsted soil diluted with varying quantities of eelworm-free soil of the same type, in differently sized pots. The rates of infestation chosen were 2.2, 0.5 and 0.14 cysts per gm. soil with a mean larval content of 58.6 larvae per cyst—a ratio of approximately 16 : 4 : 1 in initial infestation. The pots used were 10-in., 8-in. and 4-in. diameter. Half of the plants were transplanted at breakthrough to eelworm-free soil in 6-in. pots, the remainder were allowed to grow to maturity undisturbed. Replication was three-fold. At the end of the experiment soil from all the pots was washed and the cysts recovered. For plants grown to maturity in different sized pots without transplanting, the following infestation densities were recorded at the end of the experiment for different initial infestations. The data are given as cysts per gm. of soil.

Initial level	...	2.2	0.5	0.14
Final level 10-in. pots	...	4.8	4.0	1.3
Final level 8-in. pots	...	8.5	2.5	1.6
Final level 4-in. pots	...	7.1	3.8	1.7

Knowing the weight of soil contained by each size of pot, simple arithmetic shows the total yield of cysts per plant to be as follows :—

Initial level		2.2 cysts/gm.	0.5 cysts/gm.	0.14 cysts/gm.
10-in. pots	...	17,316	14,436	4,896
8-in. pots	...	14,425	5,314	3,349
4-in. pots	...	5,229	2,837	1,250

It will be seen that the total yield of cysts is directly correlated with pot size, the yield from a 4-in. pot being about one-third that from a 10-in. pot at the highest level of infestation, one-fifth at the intermediate rate and one-fourth at the lowest rate. A comparison of total cyst yields, with yields in terms of cysts per gm., is interesting. The effect of pot size in the latter case is the reverse of the former, the 4-in. pots tending to yield the greatest cyst density particularly for the highest initial rate of infestation.

The total yield of cysts from plants transplanted at breakthrough into eelworm-free soil in 6-in. pots was as follows :—

Initial level	2.2 cysts/gm.	0.5 cysts/gm.	0.14 cysts/gm.
10-in. pots ...	5,364	3,756	1,242
8-in. pots ...	3,802	1,263	1,447
4-in. pots ...	1,987	1,320	691

It will be seen that the size of pot in which the plants were grown for the first stage of their life had a marked effect on the yield of cysts, even though the environment after transplanting was uniform for all plants.

TABLE III.

Larval contents of cysts produced on plants grown in differently sized pots with different levels of infestation.

Initial infestation.		2.2 cysts/gm.	0.5 cysts/gm.	0.14 cysts/gm.
Plants grown to completion	10-in. pots	36	137	186
	8-in. pots	98	154	161
	4-in. pots	86	192	166
Plants transplanted to clean soil	10-in. pots	299	153	201
	8-in. pots	227	241	219
	4-in. pots	313	300	203

TABLE IV.

Total larval production of plants of Table III.

Initial infestation.		2.2 cysts/gm.	0.5 cysts/gm.	0.14 cysts/gm.
Plants grown to completion	10-in. pots	623,376	1,977,732	900,656
	8-in. pots	1,805,650	818,356	539,189
	4-in. pots	449,694	544,704	207,500
Transplants	10-in. pots	603,836	574,668	249,642
	8-in. pots	863,054	304,383	316,893
	4-in. pots	621,931	396,000	140,273

Larval estimations were carried out on all cysts recovered in this experiment and the results are set out in Table III. The data in this table multiplied by the corresponding yield of cysts per plant give a measure of the total number of larvae per plant at the end of the season. These data are presented in Table IV.

It is considered that several aspects of this experiment merit some consideration. There is first, the influence of pot size on yield of cysts. When plants were grown to maturity in infested soil the number of cysts produced per plant was related directly to pot size, the larger pots yielding more cysts than the smaller. This result can reflect either the varying amounts of root growth in the different sized pots or, alternatively, the varying total mass of infective material held by different sized pots at the start of the experiment. Although the former undoubtedly has some effect, the evidence generally, suggests that the latter is the more important factor. If final infestation be expressed as cysts per gm. of soil instead of as cysts per plant then it will be seen that the final intensity of infestation is no greater in larger than in smaller pots—in fact, there are indications that the reverse is the case ; the multiplication rate does not appear to be markedly influenced by pot size. Moreover, when plants are grown in different sized pots and transplanted at breakthrough into 6-in. pots of eelworm-free soil, the final total yield of cysts was greater for those plants started off in large rather than in small pots ; any differences in the final cyst count could only be a reflection of different intensities of root infestation at the time of transplanting since subsequent treatment was uniform whatever the original pot size.

Comparison of cyst yields for different original levels of infection for plants grown to maturity without transplanting, show that for 10-in. pots the ratio of cysts per gm. for the highest and lowest infestation was approximately 3 : 1 although the initial ratio was 16 : 1. Corresponding figures for 8-in. and 4-in. pots were 6 : 1 and 4 : 1 respectively. For the transplanted potatoes the ratio of yields was 4 : 1, 3 : 1 and 3 : 1 respectively for the three pot sizes. This seems to indicate that unless the initial infestation is very light, multiplication of potato-root eelworm does not follow any simple arithmetic rule. In conjunction with the data of Chitwood and Feldmesser (1949) it is obvious that as eelworm populations rise in density their multiplication rates decrease so that forecasts on population increases based on such assumptions as the compound interest law should be accepted with caution. This effect is even more apparent if the data for larvae per cyst be examined, the number of larvae per cyst is lower for plants grown in heavy infestations than for plants grown in light : if final infestation be measured by the total larval production per plant then in 10-in. pots more larvae were produced by plants grown in lightly infested soil than by those grown in heavy infestations. In 8-in. pots

the ratio of larval production for heavy : light infestations was 3 : 1 and for 4-in. pots 2 : 1.

Summary and Conclusions.

1. When potatoes are planted in eelworm infested land, the root diffusate produced results in 70—80 per cent. of the larvae emerging from the cysts in the first 4—5 weeks of growth and this effect is shown up to a distance of 6 in. from the root centre.

2. Moderate or heavy soil infestations of potato root eelworm result in a considerable root invasion prior to potatoes breaking through; several thousand cysts can result from this early attack and can cause severe dislocation of the young root.

3. The rate of multiplication of potato-root eelworm within roots is very variable and depends on the initial degree of infestation ; high initial infestations result in low multiplication rates, and low initial infestations favour high multiplication rates ; computations on population build up based on empirical relations such as " compound interest " consequently become virtually valueless.

4. Results from pot experiments should be interpreted very cautiously since pot size can have a profound influence on populations of potato-root eelworm ; extrapolation from such results is unjustified.

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*On a Remarkable New Cestode, *Meggittina baeri* gen. et sp. nov. (Anoplocephalinae) from Rodents in Southern Rhodesia.

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This highly distinctive worm was collected in Honde, Southern Rhodesia, on two separate occasions, from hosts designated " house rat " and " native granary rat " which the writer understands could readily be one and the same, since houses and granaries in these areas are built in fairly close proximity. If, however, it is assumed that these buildings are at a distance from each other, it is possible that two different genera and species are concerned ; and if such is the case, the " granary " rat might possibly be a field rat. It is regretted that the identification of the host cannot be more explicit.

The material, which was made available to the writer from the Department of Parasitology, consisted of five worms collected from the small intestine of a " house " rat from Honde mission school of 30th October, 1930, all mounted in toto ; and a further collection of about a dozen specimens taken from the small intestine of the " granary " rat at Honde on 1st November, 1930, and preserved in glycerin-alcohol.

Figures 1–4 show that the strobila of this worm appears, at least superficially, to be entirely different from that of a typical cestode. In the latter, the strobila usually consists of scolex, neck and several

* Part of a Thesis approved by the University of London for the award of the Ph.D. Degree.

segments, and is dorsoventrally flattened and elongated in the antero-posterior direction. Even in worms in which the segments are considerably broader than long, they are numerous and combine to form a typical tapeworm strobila, e.g. *Anoplocephala* species ; and in cases where the segments are few in number, e.g. *Catenotaenia oranensis*, Joyeux and Foley, 1930, the strobila is antero-posteriorly elongated. By contrast, the present specimens consist only of scolex, neck and, at the most, two segments which are enormously broad, the breadth being about 10 times the length. This curious morphology might be interpreted on the following hypothesis. If one were to take a hypothetical cestode consisting of a scolex, neck and very few segments with marginal genital pores and with numerous testes arranged in two lateral fields on either side of the female organs, and pull it laterally outward to its maximum extent, the result would be that the breadth of the strobila would be increased out of all proportion to the length, and the segments would become extremely narrow. Structures situated laterally, like the testes and genital pores, would now occupy an anterior position, and median structures like the ovary and uterus would be stretched along the breadth of the segment posterior to the testes, while the scolex would become median in position, having been drawn towards the middle of the transversely elongated strobila.

Family : Anoplocephalidae Cholodkovsky, 1902, *emend.* Fuhrmann, 1907.

Subfamily : Anoplocephalinae Blanchard, 1891, *emend.* Fuhrmann, 1932.

Meggittina baeri, gen. et sp. nov.

The worms are very thin and delicate, almost membranous in appearance and in the unmounted material, there is a tendency to twist spirally about the very long transverse axis. The length, measured antero-posteriorly from the scolex backwards, is fairly constant and varies from 1 mm. in most of the specimens to a little over 2 mm. in the largest specimen. The majority of the strobilae, with an occasional exception, e.g. as seen in Fig. 2, show a more or less pronounced upward curve towards the middle, in the region of the

Abbreviations used in Figs. 1–8.

c.s.=cirrus sac. ; **g.p.**=genital pore ; **n.**=neck ; **ov.**=ovary ; **ovd.**=oviduct ; **r.s.**=receptaculum seminis ; **sc.**=scolex ; **seg. 1**=1st segment ; **seg. 2**=2nd segment ; **s.g.**=shell gland ; **t.**=testes ; **ut.**=uterus ; **vag.**=vagina ; **v.d.**= vas deferens ; **v.g.**=vitelline gland.

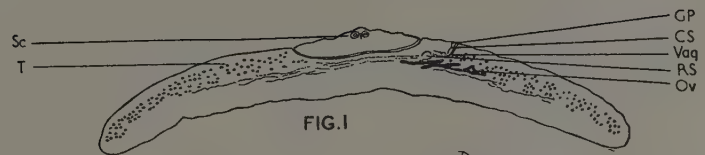


FIG.1

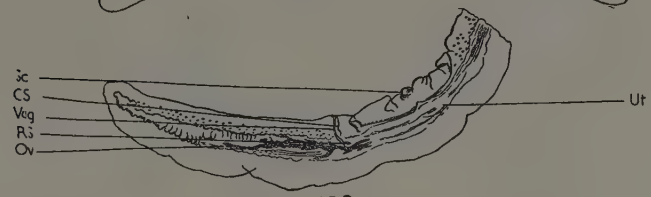


FIG.2

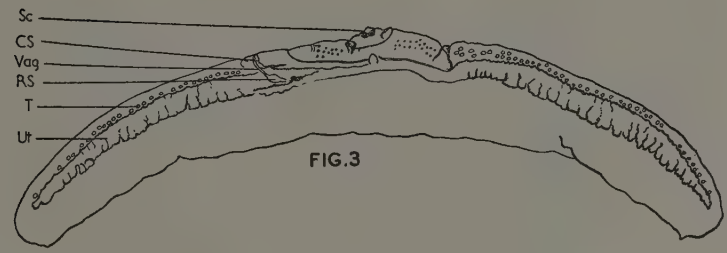


FIG.3

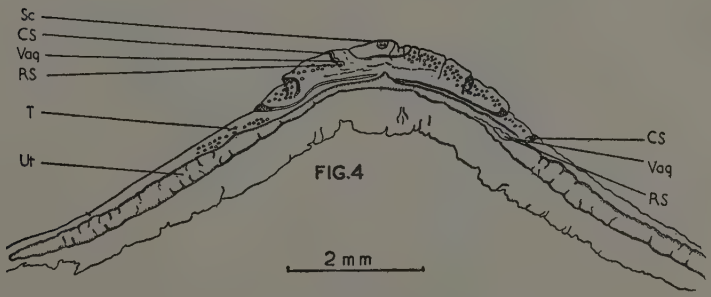


FIG.4

Meggittina baeri, gen. et sp. nov.

Fig. 1. Strobila consisting of scolex, neck and one mature non-gravid segment. Fig. 2. Strobila (incomplete) with atypical curvature, consisting of scolex, neck and one gravid segment. Fig. 3. Strobila consisting of scolex, neck, one immature segment and one gravid segment. Fig. 4. Strobila (incomplete) consisting of scolex, neck, one mature segment and one gravid segment.

scolex so that they appear to be shaped somewhat like blunt arrow-heads. The breadth is very great, varying from 8 mm. to 21 mm. in complete strobilae, and 6 mm. or a little more or a little less, in incomplete ones. It may therefore be seen that the breadth is approximately 10 times the length.

The *scolex* is an inconspicuous organ embedded in the centre of the strobila and measures 160—170 μ long by 220 μ broad. It is divided into four rather indistinct lobes by shallow grooves ; each lobe bears a rounded, unarmed sucker measuring 90—100 μ long by 100 μ broad, and the apex forms a slight, blunt projection, but there is no trace of a rostellum.

Owing to the twisted condition of the worms it was not possible to obtain any satisfactory sections, with the result that the writer is unable to supply any details of the excretory system.

The *musculature*, as far as could be ascertained consists of a readily distinguishable subcuticular zone, and a less readily distinguishable parenchymal zone, the longitudinal muscles forming a thin band composed of small bundles of fibres. Transverse and dorsoventral muscles were not seen.

On the evidence available at the moment it would appear that the *genital pores* alternate regularly, but as this condition was seen only in one strobila consisting of two segments (Fig. 4 and Fig. 5) this statement remains open to question until further material and information are obtainable. All the remaining strobilae consist of one segment only (Figs. 1, 2 and 7), except for a single strobila of two segments which, however, does not show any genital pore in the young first segment (Fig. 3). Owing to the transversely elongated condition of the strobila, the genital pore lies, like the scolex, on its anterior margin, and on account of its proximity to the scolex, it may be said to occupy a very anterior position on the margin of the segment.

The *testes* are rounded or oval bodies measuring from 70—90 μ in diameter, and are very numerous, the numbers varying between 250 and 350 in each segment. They are situated anterior to the female organs, and are separated by them into well-defined fields, and are slightly more numerous on the aporal side than on the poral. The *cirrus sac* is differentiated into two regions, a distal region which is narrow and tubular and opens almost at right angles to the margin of the segment, and a proximal region which turns inwards towards the middle of the segment at an angle to the distal region and has a larger diameter. The

Meggittina baeri, gen. et sp. nov.
Fig. 5. Central portion of strobila in Fig. 4 under higher magnification. Fig. 6. Eggs.
Fig. 7. Strobila (incomplete) showing further details of female organs.

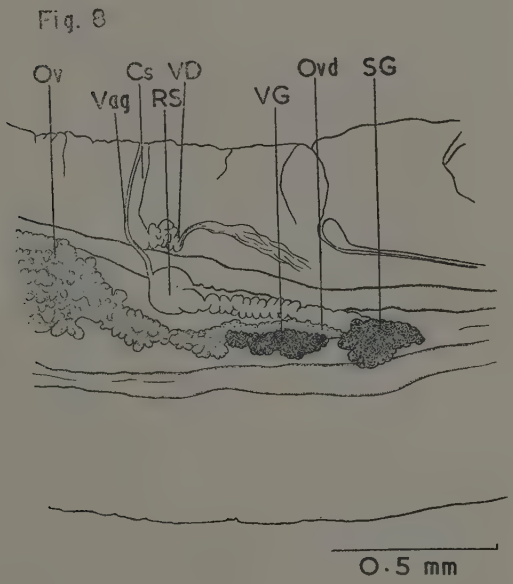
total length of the cirrus sac is 290—310 μ and the breadth is 40 μ in the distal region and 60 μ in the proximal. The *vas deferens* forms a number of coils outside the cirrus sac, but there is no external or internal vesicula seminalis.

The *female organs* are posterior to the testes, and occupy a median position in the segment. The *ovary* is poral in position and is a highly-branched organ, its branches extending for some distance into the lateral part of the segment. Towards its inner or proximal end a wide tube, the *oviduct*, is given off towards the middle of the segment and ends in an irregularly shaped organ, the *shell gland*. Posterior to the oviduct, and in close proximity to it is the compact, follicular *vitelline gland*. The *vagina* commences as a slender tube consistently posterior in position to the cirrus sac, and closely following its outer curve until its proximal end is reached when it continues in tubular form for a short distance, then suddenly expands into a large *receptaculum seminis*. This organ may be rounded or ellipsoidal, but it is characterized by being divided by a constriction into a large distal chamber and a considerably smaller proximal one from which the vagina now continues as a much wider coiled tube ending in the shell gland. The *uterus* is highly characteristic in all these worms, consisting of a very reduced median stem with an anterior pocket projecting forward in direct line with the scolex. On either side a pair of more or less well-defined lateral branches extend outwards through the breadth of the segment. When these branches reach the lateral portions of the segment they give off numerous short wide secondary branches, but always from the inner side only, the anterior and posterior margins of the uterus remaining consistently smooth. This type of branching results in the secondary branches becoming very closely crowded together, but they do not become confluent with each other, as could be distinguished from some of the horizontal sections. The *eggs* measure 20 μ and consist of an outer and an inner shell enclosing an embryo which measures 8—12 μ. A pyriform apparatus is absent.

Discussion.

The relationships of this worm would appear to lie with the Anoplocephalidae in general by virtue of its non-rostellate unarmed scolex, its numerous testes and small eggs; and with the Anoplocephalinae in particular because of its persistent uterus in the form of a transverse lobed sac. Amongst the genera and species of the Anoplocephalinae it most resembles *Catenotaenia oranensis* Joyeux and Foley, 1930.

There has been some difference of opinion on the systematic position of the genus *Catenotaenia*. It was erected by Janicki (1904) to accommodate Goeze's (1782) *Taenia pusilla* and *Taenia dendritica* with the former as the genotype. *Catenotaenia pusilla* (Goeze, 1782) was first recorded from a house mouse in Germany and since then it has been reported from various rodents in Europe, North America and Japan.



Meggittina baeri, gen. et sp. nov.

Fig. 8. Portion of strobila in Fig. 7 under higher magnification.

It has had a checkered career, being placed in various families according to the importance attached to various characteristics by the authors concerned. Meggitt (1924) placed it in the Taeniidae, and diagnosed the family as " usually " having a well-developed rostellum " usually " armed with a double crown of hooks ; with a bilobed ovary posterior to the testes, " except in *Cladotaenia* and *Catenotaenia* " and " eggs with a

thick radially striated inner envelope (except in *Cladotaenia* and *Catenotaenia*)." Joyeux and Baer (1945) recognise the similarity in the shape of the uterus of *Taenia* and *Catenotaenia* but remark that, of all the known species of *Taenia*, *T. saginata* Goeze is the only one possessing an unarmed scolex and an apical organ, and that it is a true species of *Taenia* which has undergone mutation involving the loss of the hooks, only the rostellum persisting. It appears, therefore, that *Catenotaenia* occupied a rather uncomfortable position in the family Taeniidae.

This genus was also placed in the family Dilepididae by Fuhrmann (1932) and Joyeux and Baer (1936) on account of the similarity in the arrangement of the genitalia, particularly the pre-testis position of the ovary. On the other hand, it is exceptional to find in the Dilepididae any genera with an unarmed scolex or a weakly developed rostellum, except in the subfamily Paruterininae the anatomy of whose members, however, are entirely different to that of *Catenotaenia*. It would appear, therefore, that the position of *Catenotaenia* was as uneasy in the Dilepididae as in the Taeniidae.

On the face of these arguments, Joyeux and Baer (1945) came to the conclusion that it would be best placed in the family Anoplocephalidae, and in the subfamily Anoplocephalinae on account of the unarmed scolex and absence of a rostellum which are fundamental characteristics of this family. Other similarities lie in the arrangement of the longitudinal muscles, the type of excretory system which ramifies more or less according to the species, and the arrangement of the genitalia. The differences lie in the shape of the segments which are broader than long in the majority of the Anoplocephalidae except *Oochoristica* where the mature segments are square and the gravid ones are longer than broad, and in the structure of the young uterus of *Catenotaenia*. This organ differs in *Catenotaenia* from that of any other cyclophyllidean tapeworm by appearing in its definitive form very early in development, i.e. in the form of a median axis with lateral branches, and never occurs as a single median axis as in *Taenia* where the lateral branches are only formed under the pressure of the accumulated eggs (Joyeux and Baer, 1945). However, these two authors do not feel that the condition of its uterus excludes *Catenotaenia* from the Anoplocephalinae in which the uterus is very variable being tubular, sac-like or net-like. The structure of the egg and the small size of the embryo also link *Catenotaenia* with those Anoplocephalidae in which the pyriform apparatus is absent. Baer recalls that in 1927 he suggested that the Anoplocephalidae were probably evolved from Ichthyotaeniidae parasitic in fishes and reptiles.

and points out that the presence of an apical sucker in the larval *Catenotaenia* supports this theory, since an apical organ also appears in those Ichthyotaeniidae whose life histories are known. In view of this evidence Joyeux and Baer (1945) felt justified in the inclusion of *Catenotaenia* in the family Anoplocephalidae and in particular in the subfamily Anoplocephalinae. Accordingly they proposed a new diagnosis for the genus : " Anoplocephalinae in which the scolex of the young worms bears a functional apical sucker which disappears in the adults. Excretory system with ramifications and secondary anastomoses. Genital pores alternating irregularly. Genital ducts usually dorsal to the excretory canals. No internal or external vesicula seminalis. Vagina surrounded by a sleeve of glandular cells, sometimes very long and rolled upon itself, ending in a receptaculum seminis. Testes behind, more rarely at the sides of, the female organs. Ovary very branched, forming two voluminous lobes, one ventral and the other dorsal. Vitelline gland equally branched situated in the poral half of the segment. Uterus from its beginning consists of a median stem with lateral branches. Eggs small, without a pyriform apparatus. Larva a merocercoid in mites. Adult in rodents. Type species : *Catenotaenia pusilla* (Goeze, 1782)." It is their opinion, however, that the present concept of cyclophyllidean classification may have to undergo modification when more life cycles are known, and that at present, any classification cannot be other than provisional.

Wardle and McLeod (1952) have removed *Catenotaenia* from the Anoplocephalidae and established it in a separate family, the Catenotaeniidae, with the single genus *Catenotaenia*, on the grounds that strong arguments can be raised for and against its inclusion in either the Taeniidae, Dilepididae, or Anoplocephalidae. It seems to them that it is in fact closer to the ancestral stock from which these three families divergently evolved ; therefore they consider that the creation of a separate family for it is entirely justified. It appears to the writer that the diagnosis given by Wardle and McLeod of the family is, in effect, identical with the generic diagnosis given by Joyeux and Baer. In view of the fact that no new evidence is brought forward for the establishing of a new family, only a tentative opinion expressed as to the probable evolution of the genus, the writer does not consider that there is any justification for removing *Catenotaenia* from the Anoplocephalidae. The arguments of Joyeux and Baer are more convincing than Wardle and McLeod's, and the writer agrees with the former that it is necessary to wait until more life-cycles become known in order that clear-cut relationships can be established. Therefore, in the present state of

knowledge, it seems preferable to retain *Catenotaenia* in the family Anoplocephalidae, and in the subfamily Anoplocephalinae, and it is on this basis of classification that the writer now attempts to establish the relationships of the cestode described herein and named *Meggittina baeri*.

Relationships.

That *M. baeri* is related generally to the Anoplocephalidae and particularly to the Anoplocephalinae was apparent from the start. But its generic and specific affinities were not clear until they were indicated by Professor J. G. Baer as approximating to *Catenotaenia oranensis*, Joyeux and Foley, 1930, when he examined the writer's slides on the occasion of his visit to London in 1952. He very kindly sent the writer a slide of the co-type of *C. oranensis* thereby making possible a comparison of actual specimens. *C. oranensis* Joyeux and Foley, 1930, was first described from the rodent *Meriones shawi* in Oran, Algeria, but as the description was based upon fragmented specimens it is not entirely satisfactory. It was redescribed and figured by Joyeux, Baer and Gaud (1951) on material from the same host and it is from this description combined with a study of the co-type slide that the writer is able to compare her specimens with *C. oranensis*. The relationship between the two worms is undoubted, and one immediately recognises a characteristic feature of the uterus common to both ; this is the anteriorly projecting pocket which is given off from the median stem of the uterus. Besides this outstanding feature other similarities exist. Both are composed of only a few segments, *C. oranensis* consisting of five or six segments and *M. baeri* of not more than two. Both worms are thus very short, *M. baeri* being extremely short since it does not exceed 2 mm. The length of *C. oranensis*, stated to be 60 mm. by Joyeux and Foley (1930) and 60—80 mm. by Joyeux, Baer and Gaud (1951) appeared to the writer to be excessive for worms consisting of so limited a number of segments, particularly as those segments are not abnormally long ; on enquiring of Professor Baer, he confirmed that these measurements were incorrect and suggested that the writer should make a statement to this effect. It is therefore recorded here that the length of *C. oranensis* as measured from the co-type is approximately 16 mm. and not 60—80 mm. as stated by the previous authors. From these measurements it can be seen, that macroscopically, the strobilae of *C. oranensis* and *M. baeri* do not resemble each other at all.

Internally, the two worms show resemblances and differences in structure which will be enumerated below. In both, the testes are separated into two distinct fields by the female organs, but in *C. oranensis* they are lateral in position, being situated on either side of the female genitalia whereas in *M. baeri* they are anterior to the female genitalia. In this respect the worm differs, not only from *C. oranensis* but from all other species of *Catenotaenia* in which the testes lies behind, or partly behind and at the sides of the female genitalia. In both worms, the genital pore is very anterior in position and is regularly alternating, but whereas the opening of the vagina posterior to the cirrus sac is constant in *M. baeri*, it is variable in *C. oranensis*. The cirrus sac differs in the two worms, that of *C. oranensis* being of the usual type and not showing a differentiation into distal and proximal regions as seen in *M. baeri*.

The female genitalia of the two worms bear a close resemblance to each other in general as regards arrangement, and in particular as regards the uterus. In both of them the female organs are situated between the two testicular fields, but whereas the ovary is median in *C. oranensis*, it is somewhat to one side of the median axis and may be said to be poral in position in *M. baeri*. The receptaculum seminis is ellipsoidal in the former, but is divided by a constriction into unequal proximal and distal parts in the latter. The greatest similarity between the two worms lies in the uterus ; in both a highly characteristic out-pocketing of the median stem is present which pushes forward the posterior margin of the preceding segment. This structure appears to be peculiar to *C. oranensis* and *M. baeri*, as it is not seen in any of the other known species of *Catenotaenia*. In *C. pusilla* the gravid uterus possesses a slight forward projection of the median stem which is more or less pronounced according to the state of contraction of the segment, but it never forms an indentation on the posterior margin of the preceding segment. As a natural consequence of the difference in shape of the strobilae of *C. oranensis* and *M. baeri*, the shape of the uteri also differs. In the former the uterus consists of a median stem with 9 to 10 lateral branches on each side and the characteristic anterior out-pocketing of the median stem already mentioned ; in the latter, the median stem is extremely reduced and two wide lateral branches are given off on each side, and these give off short secondary branches on their internal sides. The size of the eggs is similar in the two forms, being 18 μ in *C. oranensis* and 20 μ in *M. baeri*. The salient points of comparison and contrast between the two worms are given in Table I.

It is apparent from the discussion that the affinities of the *M. baeri* clearly lie with *Catenotaenia oranensis*. Joyeux and Foley, 1930, and it can be appreciated that the former could be derived from the latter by undergoing such modifications as a reduction in the number of segments, combined with a tremendous transverse elongation of the strobila. As

TABLE I.

	Catenotaenia oranensis Joyeux and Foley, 1930.	*Meggittina baeri*, gen. et sp. nov.
Length :	16 mm.	1—2 mm.
Breadth :	4 mm.	8—21 mm.
No. of segments :	5 or 6.	Not more than two.
Genital pore :	Anterior ; alternates fairly regularly.	Anterior ; alternates regularly.
Testes :	Very numerous (over 400). In two lateral groups on either side of female genitalia.	Very numerous (250–350). In two groups anterior to the female genitalia.
Cirrus sac :	Elongated. No regional differentiation.	Differentiated into narrow distal and wider proximal regions
Vagina :	Varies in position, anterior or posterior to cirrus sac.	Constant in position, posterior to cirrus sac.
Female organs	Separate testes into lateral groups. Receptaculum seminis ellipsoidal.	Posterior to the testes. Receptaculum seminis differentiated into two unequal proximal and distal parts.
Uterus :	Median stem directed antero posteriorly, with forwardly projecting pocket.	Median stem extremely reduced, but anteriorly projecting pocket well defined.
	9—10 lateral branches on each side.	Two wide lateral branches on each side.

the matter stands, however, one is dealing with two well-defined forms resembling each other in certain striking features, but differing as markedly in others. It appears that its very characteristic strobila separates this new cestode from other genera of the Anoplocephalinae, while the unvarying position of its testes, anterior to the female genitalia, separates it from *Catenotaenia*. The writer therefore feels justified in separating this worm from other members of the Anoplocephalinae and placing it in a genus and species of its own, namely, *Meggittina baeri*.

The generic diagnosis is as follows : Anoplocephalinae with a very reduced strobila consisting of head, neck and not more than two segments. With segments very much broader than long. Genital pores alternating regularly. One set of genitalia per segment. Cirrus sac differentiated into proximal and distal parts, without an internal or external vesicula seminalis. Testes very numerous, in two distinct fields, anterior to the female genitalia. Ovary highly branched ; vitelline gland compact. Receptaculum seminis differentiated into unequal proximal and distal parts. Uterus with very reduced median stem produced forward to form an anteriorly projecting pocket which indents the posterior border of the preceding segment ; with only two wide lateral branches on each side, giving off from their inner sides short wide secondary branches. Eggs without a pyriform apparatus. Type species : *baeri*, with the characteristics of the genus. Parasitic in rodents. Type specimens are deposited in The Department of Parasitology, London School of Hygiene and Tropical Medicine.

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On the Nematode and Trematode Parasites of some Small Mammals from the Inner Hebrides.

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The study of the parasites of small mammals of the British Isles has received little attention. A survey was made of the parasites of a wild mouse population at Oxford by Elton, Ford and Baker (1931), the identification of the parasites being made by Dr. H. A. Baylis. Elton (1934) made a brief survey of the parasites of mice on the Isle of Lewis. Other work consists of isolated references to individual species of parasites (Morgan, 1928, Baylis and King, 1932).

The present work is the result of examinations of mice and shrews from the islands of Raasay, Ulva and Scalpay in Scotland. These are situated in the Inner Hebrides, Raasay and Scalpay near the Isle of Skye and Ulva close to the Isle of Mull.

Table I.

The distribution of *A. sylvaticus*, *C. glareolus*, and *S. araneus*, examined from Raasay, Ulva and Scalpay.

Species.	Raasay.	Ulva.	Scalpay.	Total.
A. sylvaticus	15	30	—	45
C. glareolus	41	13	2	56
S. araneus	46	19	7	72

Material.

The material was kindly supplied by Mr. I. J. Linn. It consisted of 173 specimens, skinned and preserved in formalin. Three species were present, *Apodemus sylvaticus*, *Clethrionomys glareolus* and *Sorex araneus*. Table I shows the number of each species obtained from each Island.

Method.

Only the body wall, musculature and alimentary canal were examined. The musculature was inspected for encysted nematode larvae, then the stomach, intestine and rectum were removed. The stomach was carefully slit open in a Petri dish containing a little water, and both the contents and the stomach wall were examined.

A similar procedure was adopted in examining the intestine but in this case small portions about an inch long were examined separately. Fixation in formalin of the whole animal made it relatively easy to separate the parasites from the gut contents, but great care was necessary to detect the smaller nematodes. Trematodes were stained with paracarmine and nematodes were cleared in lactophenol. The present paper is concerned with the trematode and nematode fauna only. The cestodes will be the subject of a separate paper.

Results of Examination.

The results of the examination are given below. The three host species are considered separately and the species of parasites are listed according to their hosts. The distribution of the parasites is discussed under a separate heading.

Parasites of *Apodemus sylvaticus*.

Forty-five specimens of *Apodemus sylvaticus* were examined, 30 from Raasay and 15 from Ulva, none being available from Scalpay. Eleven of the hosts examined contained no parasites.

Trematodes.

Two species of trematodes were found :

Lyperosomum vitta (Duj., 1845) and *Brachylaemus recurvum* (Duj., 1845).

Lyperosomum vitta (Duj., 1845).

A single specimen was found in the upper small intestine of a male *Apodemus sylvaticus* from Ulva. The species is not common but has been recorded by Dujardin (1845) from *Apodemus sylvaticus* in France, and by Elton (1931) and Baylis (1928) from *Apodemus sylvaticus* at Oxford. The specimen recorded by Elton has been described by Baylis (1927) in a separate paper. The specimens described by Dujardin and Baylis were incomplete. The specimen obtained in the

present work, though complete, is in two pieces and badly fixed. Thus, the length of a complete specimen has not been recorded, but is given by Dujardin as greater than 10 mm. and by Baylis as greater than 6 mm., these figures being the length of the largest fragment available in each case. Assuming that the two pieces obtained in the present work represent the whole specimen, the length is approximately 7 to 7.5 mm., rather less than that suggested by Dujardin. This specimen agrees closely with that described and figured by Baylis but is slightly smaller, as will be seen by reference to Table II.

TABLE II.

Comparison of the measurements of *L. vitta* from Ulva and Oxford.

Character.	*L. vitta* (Ulva).	*L. vitta* (Oxford).
Length	7.0–7.5 mm	>6 mm
Maximum width	0.70 mm	1.1–1.3 mm
Oral sucker	0.22 mm	0.30–0.31 mm
Ventral sucker	0.31 mm	0.40–0.45 mm
Cirrus sac		
Length..	0.16 mm	0.30–0.35 mm
Diameter	0.07 mm	0.10–0.14 mm
Testis :		
Length..	0.15 mm	0.23–0.30 mm
Diameter	0.22–0.23 mm	0.30–0.47 mm
Vitelline glands :		
Length..	2.4–2.5 mm	No record
Egg :		
Length..	0.040 mm	0.045 mm
Diameter	0.022 mm	0.025 mm

Brachylaemus recurvum (Duj., 1845).

Baylis (1927) reported that this species is more common than *L. vitta*. It has been recorded by Dujardin (1845), Elton (1931) and Baylis (1927, 1928). In the present work the species was found in one specimen from Raasay, a group of five parasites being found in the small intestine. These specimens agree closely with those described by previous workers (Dujardin, 1845, Baylis, 1927).

NEMATODES.

Three species of nematodes were recorded from *A. sylvaticus*, one of these, *Heligmosomum glareoli* (Baylis, 1928) being a new record for

this host. The distribution of the species on the islands is recorded in Table III.

TABLE III.

The distribution of nematodes in *A. sylvaticus* on the islands of Raasay and Ulva.

Species.	Raasay		Ulva	
	No.	%	No.	%
Capillaria muris-sylvatici	4	26.5	13	43.5
Heligmosomum glareoli ..	14	93.5	17	56.5
Syphacia stroma	1	6.5	2	6.5
No nematodes	1	6.5	10	33.5
Total number of hosts examined	15	—	30	—

Capillaria muris-sylvatici (Diesing, 1851).

This species is a common parasite of *A. sylvaticus* and has been reported from Oxfordshire (Elton, 1931, Baylis, 1939) and Westmorland (Baylis, 1939). In the present work this species was found in the stomach of 17 out of 45 specimens examined, but only small numbers of the parasite were obtained.

Syphacia stroma (Linst., 1884).

This species has been reported from *A. sylvaticus* (Morgan, 1932 and Baylis, 1939). In the present work this species was found to be very abundant, being found in 31 out of 45 specimens examined. The number of parasites per host was high, in most cases more than 100 were present, and in a small number of cases more than 1,000 were obtained from a single host. A large number of the parasites were females, the males forming less than 5 per cent. of the total. The parasites were concentrated in the small intestine, few being found in the large intestine.

Heligmosomum glareoli (Baylis, 1928).

This species is a common parasite of *Clethrionomys* (Baylis, 1928 and Elton, 1931), but hitherto has not been reported from *A. sylvaticus*. Three out of 45 *A. sylvaticus* examined harboured this parasite, specimens being recorded from Raasay and Ulva. Only one specimen was obtained from each of the infected hosts, whereas, as will be seen below, the number found was much greater in the usual host, *C. glareolus*.

Parasites of *Clethrionomys glareolus*.

Fifty-six specimens of *Clethrionomys glareolus* were examined, 41 from Raasay, 13 from Ulva and 2 from Scalpay. Only two specimens were completely free from nematodes. No trematodes were found, and none have been recorded previously from this host.

Nematodes.

Six species of nematodes were found, 3 of these being new records for this host, and one, *Longistriata wolgaense*, being a new record for this country. The incidence of infection was very high, 100 per cent. of the specimens from Raasay and 97.5 per cent. from Ulva, harbouring nematodes. The most abundant species were *Capillaria muris-sylvatici* and *Heligmosomum glareoli*. The distribution of the nematodes is given in Table IV.

Table IV.

The distribution of nematodes in *C. glareolus* on the islands of Raasay, Scalpay and Ulva.

Species.	Raasay		Ulva		Scalpay	
	No.	%	No.	%	No.	%
Capillaria muris-sylvatici	33	80	2	15.5	1	
Trichuris muris	0	0	1	7.5	0	
Trichostrongylus retortaeformis	4	10	1	7.5	0	
H. glareoli	31	75	7	54	1	
Longistriata wolgaense ..	1	2.5	7	54	0	
Aspiculuris tetraptera ..	0	0	3	23	0	
No nematodes	1	2.5	0	0	1	
Total number of hosts examined	41	—	13	—	2	

Capillaria muris-sylvatici (Diesing, 1851).

This species was abundant in *C. glareolus* from Raasay, but was much less common in Ulva and Scalpay. In many cases large numbers of the parasite were present embedded in the wall of the stomach. This species has been recorded from *C. glareolus* by Baylis (1928) and Elton (1931).

Trichuris muris (Schrank, 1788).

A single female of *T. muris* was found in the large intestine of a

specimen of *C. glarcolus* from Ulva. This is a new host record for *C. glareolus*, previous records being from *Apodemus sylvaticus* in Europe (see Hall, 1916) and from " mice " from Paris (Brumpt, 1936).

Trichostrongylus retortaeformis (Zeder, 1800).

This species is a common parasite of rabbits and hares in Great Britain. This is, however, the first record of its occurrence in *C. glareolus*. The parasite was obtained from specimens from Raasay and Ulva islands, in all cases from the large intestine and rectum of the host. This is a new host record for the species. It is interesting to note that these specimens were mature, although smaller than the species from the rabbit, and that they developed in the large intestine and rectum, whereas they are found in the small intestine of the normal host.

Heligmosomum glareoli (Baylis, 1928).

This species was found in the small intestine. It was found most frequently in specimens from Raasay, where 75 per cent. of the specimens examined were infected. The species has been recorded from this host by Baylis (1928) and Elton (1931).

Longistriata (*Longistriata*) *wolgaense* (Schul'ts, 1926).

Male: length 1.8–2.0 mm.; diameter 0.05 mm.
Female: length 2.0–2.2 mm. ; diameter 0.05 mm.

Description of Material (Figs. 1–2).

The description of the specimens found is as follows :—

Small worms, coiled in a loose spiral. The cuticle bears feebly developed longitudinal ridges with fine transverse striations. Cephalic inflation small or absent ; when present it is not separated from the rest of the body cuticle.

Male: Bursa relatively small, symmetrical, 0.08 mm. long and 0.085 mm. wide. The bursa always appears to be partly closed and can only be unrolled with great difficulty. The ventral rays arise from a common trunk and diverge, both curving anteriorly and being widely separate from the lateral rays. The antero-lateral and medio-laterals arise from a common trunk, being close together except at the tips where they diverge sharply. The postero-lateral arises at the base of the medio-lateral but diverges sharply from it, curving posteriorly.

The dorsal ray, 0.04 mm. long, has a short, stout trunk and is divided in its distal half into two slender branches ending in bifid points. The externo-dorsals arise just before the division of the dorsal ray and run parallel with its branches. The externo-dorsals are stout compared with the branches of the dorsal ray. The spicules are long and slender, 0.30 mm. long, and bear a longitudinal striation. The gubernaculum is well developed, boat-shaped, 0.036 mm. long.

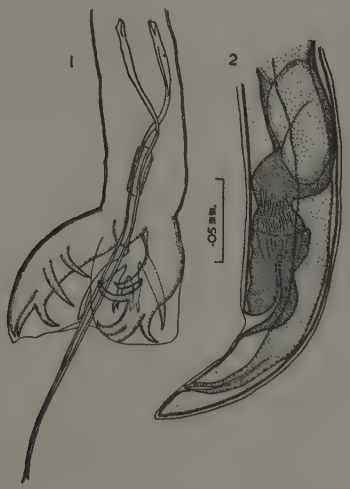

Fig. 1. *Longistriata wolgaense*. Bursa of male.

Fig. 2. *Longistriata wolgaense*. Posterior end of female.

Female: Tail conical, ending in a blunt point. Vulva and anus close together, vulva 0.08 mm. from tail. Uterus single, ovejector well developed, eggs thin shelled, 0.06 mm. × 0.04 mm.

The bursa, gubernaculum and the shape of the spicules agree with the description of *Longistriata wolgaense* (Schul'ts, 1926). The length of the spicules of *L. wolgaense* is given as 0.39 mm. compared with 0.30 mm. above, and the head of *L. wolgaense* is described as having an annulated cephalic inflation. This latter character, however, is subject to variation, and also may be affected by the method of

preservation. In the present work there is evidence that in some cases annulation is the result of shrinkage.

In view of the close agreement with regard to the bursa and gubernaculum, and the striation of the spicules, it must be concluded that the present species is *L. wolgaense*.

L. wolgaense has previously only been recorded from *Arvicola amphibius* (*Mus amphibius*) in Russia (Schul'ts, 1926), only the male having been described. Thus this a new record for this host, and a new record for this country. The female is described for the first time. In the present work the parasite was found in the small intestine of 8 *C. glareolus* from Raasay and Ulva. Up to 30 specimens were observed from each host, and it cannot be considered a rare species on these islands.

Aspiculuris tetraptera (Nitzsch, 1821).

The species was not common, occurring in only 3 animals from Ulva. Only 10—20 specimens were found in each host, specimens being taken from both the small and large intestine. The parasite has previously been recorded in this country by Baylis (1928) and Elton (1931).

Parasites of *Sorex araneus*.

Seventy-two specimens of *Sorex araneus* were examined, 46 from Raasay, 19 from Ulva and 7 from Scalpay. A greater number of species of parasites was found in this host than in either *Apodemus sylvaticus* or *Clethrionomys glareolus*. Only 7 specimens were free from trematodes and nematodes.

Trematodes.

Twenty-six specimens of *S. araneus* out of 72 examined were infected with trematodes, infected animals being found on all three islands. All the trematodes found were tentatively identified as *Brachylaemus fulvus* (Dujardin, 1843), *B. dujardini* (Baer, 1928) being considered a synonym. The systematics of the genus *Brachylaemus* are difficult, and the species have not all been adequately described. In this work, however, the trematodes were found to agree closely with the description of *B. fulvus* given by Dollfus (1935). Baylis (1928) recorded *Distoma migrans* Duj., 1845, and *Distoma exasperatum* Rud., 1819, from *S. araneus*, but these records were published before the classification of the genus *Brachylaemus* had been revised by Dollfus (1934, 1935), and

it is possible that Baylis's specimens need further examination in the light of this later work.

B. fulvus has not been reported previously from this country, therefore this is a new record for this host in Britain.

NEMATODES.

Eight species of nematodes were found, 4 of these being new records for this host, and 3 being new species. The distribution of the species on the islands is given in Table V.

TABLE V.

The distribution of nematodes in *S. araneus* on the islands of Raasay, Ulva and Scalpay.

Species.	Raasay No.	Raasay %	Ulva No.	Ulva %	Scalpay No.	Scalpay %
Parastrongyloides winchesi	25	54	8	42	4	57
Capillaria exigua	6	13	3	16	0	0
Longistriata depressa ..	6	13	2	10	1	14
Longistriata codrus ..	34	73	10	52	4	57
Longistriata trus	3	7	0	0	0	0
Longistriata didas ..	32	70	9	48	4	57
Soboliphyme soricis ..	1	2	5	26	0	0
Porrocaecum spirale? (larva)	1	2	6	31	1	14
No nematodes	4	9	3	16	1	14
Total number of hosts examined	46	—	19	—	7	—

Capillaria exigua (Duj., 1845).

A small number of *Capillaria* of this species was found in the stomachs of 9 out of 72 specimens examined, both males and females being present. The parasite has previously been recorded by Joyeux and Baer (1937) from *Sorex araneus*, and from Scotland from the hedgehog (*Erinaceus europaeus*) by Cameron (1933).

Longistriata spp.

In Great Britain only one species of *Longistriata*, *L. depressa*, has previously been recorded (Baylis, 1939) from *S. araneus*. In the present work four species were found, only one of which could be assigned to a known species. The other three species are named and described below.

The genus *Longistriata* is a complex group of the subfamily Viannaiinae (Neveu Lemaire, 1934), the systematics of which have been considered in a monograph by Travassos (1937). While the classification adopted by Travassos may not be an ideal one, it is the only comprehensive work available, and is a considerable advance on the work of Dikmans (1935), Neveu Lemaire (1934), Chandler (1932), and Schul'ts (1926). Accordingly, the scheme prepared by Travassos has been adopted, and the three new species described below are placed in the genus *Longistriata* of the subfamily Viannaiinae. Travassos divided the genus into four subgenera, separated on the characters of the spicules. The subgenus *Longistriata* is characterised by the possession of " relatively long, slender spicules, three times the body width," and on the basis of this character the three new species described have been placed in this subgenus, and have been named *L. codrus*, *L. trus* and *L. didas*. Males of four species were found but females of only three species. Because *L. caudabullata* Dikmans (1946) is considered synonymous with *L. depressa* (Duj., 1845) for reasons given below, the female of *L. depressa* could be identified from Dikmans' description. A male and female of *L. codrus* were found in copulation, and thus the two sexes of this species could be linked. There thus remained two species of males, *L. didas* and *L. trus*, and only one female. On the basis of the following figures the female was assigned to the species *L. didas*.

Numbers of *S. araneus* infected :—

(1) 33 infected with male *L. didas*.
(2) 3 infected with male *L. trus*.
(3) 30 infected with female.
(4) 18 infected with male *L. didas* and female.
(5) 1 infected with male *L. didas*, male *L. trus* and female.
(6) 1 infected with male *L. trus* and female.

Assuming that the sex ratio of the two species does not deviate greatly from 1 : 1, this distribution indicates that the unknown female is *L. didas*, not *L. trus*. Only 5 males of *L. trus* were found, no females of this species being observed.

Longistriata (*Longistriata*) *depressa* (Duj., 1845).

Male : length 1.8–2.2 mm.; diameter 0.05 mm.
Female : length 1.7–1.9 mm.; diameter 0.056 mm.
material available : 3 males, 7 females.

Description of material (Figs. 3–4).

The male is not coiled, but the female is coiled in a loose spiral typical of the Viannaiinae. The cuticle bears longitudinal ridges which are not distinct but are marked with fine transverse striations. The head has a cuticular dilation approximately 0.04 mm. wide. This cephalic dilation bears distinct transverse striations in the three males, but no striations were detected in the females. It is probable that the variation observed is the result of imperfect fixation and shrinkage.

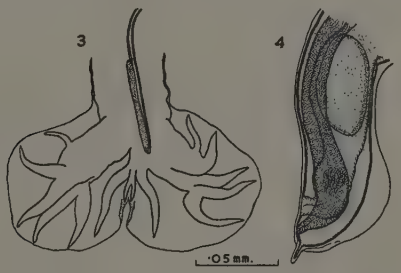

Fig. 3. *Longistriata depressa.* Bursa of male.

Fig. 4. *Longistriata depressa.* Posterior end of female.

Male: Bursa 0.08 mm. long and 0.12 mm. wide, symmetrical. The bursal membrane has a deep incision in the midline, the cleft extending down between the branches of the dorsal ray. The ventral rays arise from a common trunk and diverge, the ventro-lateral being much longer than the ventro-ventral. The antero-lateral and medio-lateral rays arise from a common trunk and diverge slightly at the tips. The postero-lateral ray arises at the base of the medio-lateral and diverges sharply from it. The lateral rays are approximately equal in size to each other and to the ventro-lateral. All the rays reach to the bursal margin, and are sharply pointed. The dorsal ray has a short, stout trunk from which the externo-dorsal rays arise. The externo-dorsals are the largest of the bursal rays, and between them the dorsal ray divides into two small, slender branches, with undivided tips. The spicules are simple, slender, 0.30 mm. long. A well-developed rod-like gubernaculum is present, 0.05–0.07 mm. long, extending into the large, elongate genital cone.

Female: Body conical posteriorly, terminating in a spike 0.016–0.020 mm. long. The cuticle has a dorsal inflation around the tail, extending to the spike. Vulva and anus are close together, vulva 0.036 mm. (average) from tail. Uterus single, ovejector well-developed 2—5 eggs present, approximately 0.060 × 0.035 mm.

The specimens obtained in this work closely resembled both *L. depressa* (Duj.) and *L. caudabullata* Dikmans. These two species were separated by Dikmans on the basis of the presence of a vesicular swelling at the posterior end of the female of Dikmans' specimens, this character not being described by Dujardin. While Baylis (1939) has recorded *L. depressa* from this host, he gave no description of his specimens.

The gubernaculum and genital cone described from the present specimens and from *L. caudabullata* are not mentioned by Dujardin in his description of *L. depressa*, and are absent from the figure of this species by Von Linstow. The absence of this character from the early description might have been taken by Dikmans as a point of specific difference. Dikmans, however, points out the close similarity between the bursa of *L. depressa* and *L. caudabullata* and seems unwilling to separate the species on the characters of the male. He takes the unusual step of referring to the female for the main specific difference, separating the two species on the basis of the absence of a posterior cuticular inflation in *L. depressa*. This cuticular inflation is, however, subject to variation as a result of preservation and mounting, and in two of seven females available the posterior swelling had collapsed. It is interesting to note that according to Travassos (1921), Dujardin's specimens were probably badly fixed. The absence of the gubernaculum from the description and figure of *L. depressa* may be related to the date at which they were published, when the systematic importance of this structure was not properly recognised. On the basis of these considerations, and in view of the close similarity between the bursas of these two species, *L. caudabullata* Dikmans is made a synonym of *L. depressa* (Duj., 1845).

L. depressa may now be redescribed as follows :—*Specific diagnosis:* Small worms, body straight or rolled in a loose spiral. Cuticle bears longitudinal ridges marked with fine transverse striations. Cephalic inflation 0.04–0.05 mm. long and 0.02–0.03 mm. wide.

Male: Bursa symmetrical, bursal membrane has a deep incision in the midline, extending down between the branches of the dorsal ray.

Ventral rays arise from a common trunk and diverge, the ventro-lateral being much larger than the ventro-ventral. Antero-lateral and medio-lateral rays arise from a common trunk and diverge at the tips. Postero-lateral ray arises from the base of the medio-lateral and diverges sharply from it. The lateral rays are approximately equal in size to each other and to the ventro-lateral. All the rays reach to the bursal margin and are sharply pointed. The dorsal ray has a short, stout trunk from which the large externo-dorsals arise, and divides distally into two small slender branches with undivided tips. Spicules simple and slender, 0.20–0.30 mm. long. Gubernaculum present, 0.05–0.07 mm. long, genital cone large and elongate.

Female: Body conical posteriorly, with a large dorsal cuticular inflation around the tail. The body ends in a bluntly rounded point, 0.015–0.020 mm. long. Vulva and anus posterior and close together, vulva 0.02–0.06 mm. from tail. Uterus single, ovejector well-developed, 2—5 eggs present, approximately 0.05–0.0 6mm. long and 0.030–0.035 mm. wide.

The bursa of the present specimens shows a striking resemblance to that of Dikmans' specimens, both in the form of the rays and in the gubernaculum and genital cone, and like Dikmans' specimens, also resembles the bursa of *L. depressa*. The spicules of Dikmans' specimens are 0.20 mm. long, those of *L. depressa* 0.24 mm. long and those of the present specimens 0.30 mm. in length. The deep incision in the bursal membrane of the present specimens is not mentioned or figured by Dikmans. However, the bursa may only be spread out with great difficulty and this incision could easily be overlooked. According to Dikmans, his specimens and *L. depressa* are the only species of the genus *Longistriata* in which the branches of the dorsal ray are undivided at their tips, and the same condition was found in the present specimens. The female of the present specimens shows a remarkable resemblance to that of Dikmans' specimens, and was in fact placed as the female of this species because of this resemblance. Thus the present specimens are assigned to the species *Longistriata* (*Longistriata*) *depressa* (Duj., 1845), of which *L. caudabullata* is a synonym.

The present specimens of *L. depressa* were found in the intestine of 9 specimens of *S. araneus* from Raasay and Ulva. Only 10 specimens of the parasite were obtained and the species must be considered infrequent on these islands.

Longistriata (*Longistriata*) *codrus*, n. sp.

Male: length 1.6–2.1 mm.; diameter approximately 0.03 mm.
Female: length 1.5–2.0 mm.; diameter approximately 0.055 mm.

Specific diagnosis (Figs. 5–6).

Small worms, body rolled in 3—4 loose spirals. The cuticle bears longitudinal ridges marked with distinct transverse striations. The head bears a cephalic inflation approximately 0.05 mm. long and 0.023 mm. wide, the head without the capsule being 0.013–0.016 mm. in diameter.

Male: Bursa relatively large, approximately 0.15 mm. long and 0.15 mm. wide, slightly asymmetrical, the right lobe being larger than the left. The right externo-dorsal and postero-lateral rays are approximately 1½ times the length of the corresponding rays on the left, the other rays being more or less equal on both sides. The ventral rays arise from a common trunk, close together, but diverge distally, the ventro-lateral being slightly larger than the ventro-ventral. The antero-lateral and medio-lateral rays arise from a common trunk, close together, but diverge slightly. The postero-lateral arises from the base of the medio-lateral and curves away from it. The postero-lateral is the largest of the lateral rays. The asymmetry of the bursa is mainly one of size, the form of the rays being the same on both sides. The dorsal ray is very long and stout, 0.10–0.13 mm. in length, forming a common trunk from which the externo-dorsals arise. These latter arise asymmetrically just below the middle of the trunk, the ray of the left lobe arising first; they are long and very slender, the ray of the right lobe being longer than the left. The dorsal ray divides distally into two relatively short divergent branches, each giving off a small spur just before the sharply pointed tip. The spicules are simple and slender, 0.16 mm. long. The gubernaculum is boatshaped, 0.04 mm. long and is slightly forked at its posterior tip. The genital cone is not large, but bears two outgrowths, one on each side, giving the appearance of a pair of horns.

Female: Body slightly stouter than that of the male, rounded posteriorly, with a large swelling of the cuticle around the tail. This swelling is much more prominent than the swelling observed in *L. depressa*. Vulva and anus are posterior and close together, the vulva 0.035 mm. (average) from the tail and opening at the beginning of the cuticular swelling. Uterus single, ovejector prominent, 3—5 eggs, approximately 0.05 mm. by 0.03 mm., present in the uterus.

Host : Sorex araneus.

Location : Intestine.

Locality : Raasay, Ulva and Scalpay.

Affinities : This species is characterised by the long dorsal ray and the slender, asymmetrical externo-dorsals. It most closely resembles *L. streptocerca* (Baylis, 1928) but may be separated from this species by the following characters : the length of the spicules ; the form of the terminal branches of the dorsal ray ; the degree of asymmetry of the bursa ; and the relative lengths of the medio-lateral and postero-lateral rays.

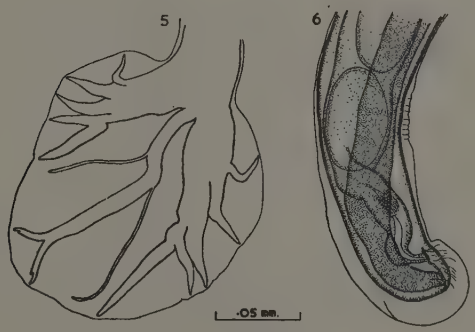

Fig. 5. *Longistriata codrus*, n.sp. Bursa of male.

Fig. 6. *Longistriata codrus*, n.sp. Posterior end of female.

This species was common, occurring in 48 out of 72 specimens of *S. araneus* examined. Males and females were found in approximately equal numbers, in the intestine of the host. Only small numbers of parasites were found, usually ranging from 1—20 parasites per host. These small numbers may be due in some measure to the small size of the parasite, which makes it difficult to detect, and may not be a true picture of the actual incidence of infection. This applies equally to the other *Longistriata* species described in this paper.

Longistriata (*Longistriata*) *trus*, n. sp.

Material: 5 males.
Male: 1.8–1.9 mm. long ; 0.05–0.056 mm. diameter.
Female: Unknown.

Specific diagnosis (Fig. 7).

Small slender worms, coiled in a loose spiral. The cuticle bears longitudinal ridges with fine transverse striations beginning just behind the head. The head, diameter 0.013–0.016 mm. has a cuticular inflation, 0.05 mm. long and 0.03 mm. wide, but there is no constriction separating this from the rest of the body cuticle.

Male: Bursa relatively large, 0.14 mm. long and 0.14 mm. wide, and slightly asymmetrical. All the rays of the left lobe are approximately 1½ times as large as those of the right, but the arrangement of the rays is symmetrical. The ventral rays arise from a common trunk, but diverge sharply, the ventro-ventral being directed anteriorly. The antero-lateral and medio-lateral rays arise from a common trunk, and are close together except at the tips. The postero-lateral arises at the base of the medio-lateral, and curves away from it posteriorly. The dorsal ray is large and stout, 0.11 mm. in length, and divides in its distal third into two stout, widely divergent branches, each bearing a small spur on the inner surface and terminating in a point. The stout externo-dorsals arise asymmetrically at about the mid-point of the dorsal trunk. The spicules are simple, and slender, 0.16 mm. in length. The genital cone is long and prominent and bears two outgrowths resembling horns. There is a rudimentary gubernaculum.

Female: Unknown.
Host: Sorex araneus.
Location: Intestine.
Locality: Raasay.

Affinities: This species can be immediately separated from *L. codrus* by the stout externo-dorsal rays. The general character of the bursa resembles that of *L. musculi*, especially in the disposition of the rays. In *L. musculi*, however, the externo-dorsals arise at the base of the dorsal trunk, whereas those of *L. trus* arise at the mid-point of the dorsal trunk. The spicules of *L. trus* end in simple points, those of *L. musculi* are dilated at the tips.

Only five specimens of this species were found in the intestine, all

of them males. Only 3 out of 72 specimens were infected, thus it was concluded that this species is rare on these islands.

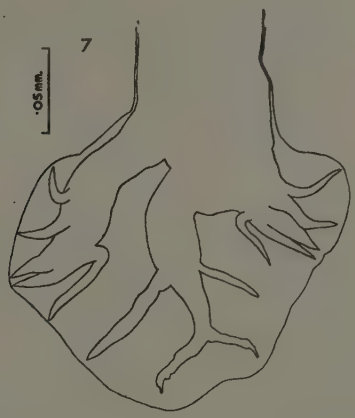

Fig. 7. *Longistriata trus*, n.sp. Bursa of male.

Longistriata (*Longistriata*) *didas*, n. sp.

Male: length 1.6–1.9 mm.; diameter 0.045–0.055 mm.

Female: length 1.8–2.3 mm.; diameter 0.06–0.09 mm.

Specific diagnosis (Figs. 8–9).

Small worms in which the body may be in one loose spiral or almost straight, with only the posterior region curved. The cuticle bears longitudinal ridges with delicate cross-striations. Head, 0.013–0.016 mm. in diameter, bears a cephalic inflation 0.045 mm. long and 0.030mm. wide which is distinctly separated from the cuticle of the body by an annular constriction.

Male: Bursa not large, 0.085 mm. long and 0.15 mm. wide. The bursa has a curious asymmetry in the right ventro-lateral and antero-lateral rays which are closely apposed throughout their length, relatively broad and terminating in a blunt point. In the right lobe the ventro-ventral arises separately and diverges from the ventro-lateral. The medio-lateral and postero-lateral rays arise from a common trunk, and

diverge sharply from the antero-lateral, the postero-lateral curving sharply to the posterior edge of the bursal membrane. The ventro-ventral, medio-lateral and postero-lateral rays of both sides are symmetrical. In the left lobe the ventral rays arise from a common trunk and diverge widely, the ventro-ventral curving anteriorly. The antero-lateral and medio-lateral rays also arise from a common trunk, diverging only in the distal half. The postero-lateral arises at the base of the medio-lateral and diverges from it sharply. The dorsal ray is large, having a short stout trunk from which arise the long, stout externo-dorsal rays. The externo-dorsals are slightly asymmetrical, the right arising before the left and being thicker at its base. The dorsal trunk continues between the externo-dorsals but is only half its previous thickness, and divides in its distal quarter into two short, stout branches which diverge at the base and then curve towards the mid-line distally. These branches bifurcate at the tips, the outer fork of the bifurcation being the larger. All the rays extend to the bursal margin. The spicules are simple and slender, 0.16 mm. long. The genital cone is small and there is no gubernaculum.

Female: Body slightly longer and stouter than the male. The posterior end of the body narrows sharply and is bluntly rounded, terminating in a short, broad spine. The vulva and anus are posterior, close together, the vulva being approximately 0.023 mm. from the posterior end. The uterus is single, with a well developed ovejector, eggs 0.057 mm. by 0.032 mm. There is no inflation of the cuticle around the tail, but the cuticle extends over the spike, which is 0.066 mm. in length.

Host: Sorex araneus.
Location: Intestine.
Locality: Raasay, Scalpay and Ulva.

Affinities.

In his generic description of the *Longistriata*, Travassos states that there is no terminal spike at the tail of the female. However, Dikmans (1946) points out that the type species *L. depressa*, may have a terminal spike as described by Dujardin (1845), and therefore the presence of such a spike does not exclude a species such as *L. didas* from this genus.

The bursa of the male of this species resembles that of *L. trus*, particularly in the structure of the dorsal ray, and was for some time confused with it. On close examination, however, it is possible to

separate the two species by the bifid branches of the dorsal ray of *L. didas*, which are also never widely divergent as are those of *L. trus*. In addition, the peculiar asymmetry of the right ventro-lateral and antero-lateral rays was found to be constant, and not as was first thought, an individual abnormality. This peculiar asymmetry appears nowhere else in the subgenus and immediately identifies this species.

L. didas was found in the intestine of 45 of the specimens examined, and is a common parasite of *S. araneus* on these islands.

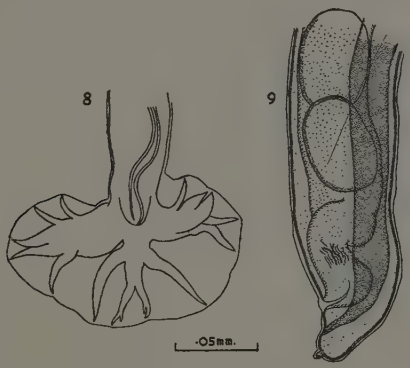

Fig. 8. *Longistriata didas*, n.sp. Bursa of male.
Fig. 9. *Longistriata didas*, n.sp. Posterior end of female.

An interesting feature of the males of species *L. codrus*, *L. trus* and *L. didas* is that in all three the spicules are only 0.16 mm. in length. In no other species of the subgenus are the spicules less than 0.20 mm. long. This cannot be related to the small size of these species as in *L. depressa*, which is the same size, the spicules are 0.20–0.30 mm. long, and in *L. eta*, Travassos, 1937, which is 1.2–1.4 mm. in length, the spicules are 0.30–0.36 mm. long.

Thus these three species *L. codrus*, *L. trus* and *L. didas* form a group within the subgenus *Longistriata*. The shortness of the spicules does not exclude these species from the subgenus, because the subgenus *Longistriata* is characterised by " simple, slender spicules which are three times the body width " (Travassos, 1937). The body width in the three species is approximately 0.05 mm., and the length of the spicules 0.16 mm., thus they may be included in this subgenus. The alternative subgenus, *Heligmonella*, in which the spicules are only twice

the body width, has spicules which are short and thick, at least three times as thick as those of the three species considered here. Thus the species have been placed in the subgenus *Longistriata*, as it was considered that the shortness of the spicules did not justify the erection of a new subgenus.

Parastrongyloides winchesi Morgan, 1928.

This species was present in the intestine of *S. araneus* from all three islands, and approximately 30 per cent. of the specimens examined harboured the parasite. Only 1—10 specimens were found in each host. The species was described by Morgan (1928) from *S. araneus* and *Talpa europaea*, and the description included an account of the parasitic male. Mature males and females were found in the present work, females being much more numerous than males. This confirms Morgan's observations on the sex ratio.

Soboliphyme soricis Baylis and King, 1932.

This species was first described by Baylis and King (1932) from a specimen of *Sorex araneus* caught at Millport. In the present work the parasite was found in only 6 out of 72 specimens examined. Only 1—3 parasites were found in each host. From one specimen of *S. araneus* from Ulva several larvae were obtained.

Porrocaecum (*spirale* ?) larva.

Encysted larvae were found on the surface of the body musculature and on the wall of the gut in eight specimens. The shape of the lips and the size of the worms make it possible to assign these larvae to the genus *Porrocaecum*. From records of the occurrence of *Porrocaecum* spp. in this country, the most probable species, based on a consideration of the habits of the host, is *P. spirale*, a parasite of owls. This species has not been recorded from *S. araneus* in this country, but it is common and has undoubtedly been found by other workers. *Porrocaecum* sp. larvae have been recorded from *S. araneus* in Russia by Pologentsev (1935).

A Comparison of the Parasites from Raasay, Ulva and Scalpay.

This comparative treatment is made difficult because of the great inequality in the numbers of specimens from the three islands. In addition, the numbers of animals caught are not necessarily representative samples of the population on these islands. The largest number, 102, was collected from Raasay, while 62 came from Ulva, and only 9 from Scalpay.

Apodemus sylvaticus.

The nematode fauna from Raasay and Ulva in this host was similar. Only three species were found and these occurred on both islands. Different species of trematodes were found, but as only one host specimen from each island was infected it is probable that examination of a larger number of specimens would have shown these species to be present on both islands. *Lepoderma* (?) *muris*, a very rare species, was not recorded.

During a survey of wild mice at Oxford carried out by Elton, Ford and Baker (1931), a large number (692) of *A. sylvaticus* were examined for parasites. The nematode fauna of the specimens from Oxford may be compared with the nematode fauna recorded in this present work. The only species common to both localities is *C. muris-sylvatici*. No specimens of *H. glareoli* were found in *A. sylvaticus* at Oxford even though *C. glareolus* from the same area harboured the parasite. In the present work only three *A. sylvaticus* were found to be infected with *H. glareoli* and in each case only one specimen of the parasite was found. This suggests that these infections were accidental and that, as at Oxford, *A. sylvaticus* is not a normal host for this species. The occurrence of infection on Raasay and Ulva may be due to a more restricted habitat in which such accidental infection would be more likely to occur. *Syphacia* spp. Elton found that 32 per cent. of the specimens examined at Oxford were infected with *Syphacia*, but reports the species as *S. obvelata*. Morgan (1932), however, distinguished two species of *Syphacia*, *S. obvelata* and *S. stroma*, the latter being confined to *A. sylvaticus*. As the *Syphacia* species from Oxford was identified before the publication of Morgan's paper it is possible that the species concerned was in fact *S. stroma*. Elton (1934) records *Syphacia stroma* (?) from *A. sylvaticus* on the Isle of Lewis, but as no males were found he was unable to establish the species concerned. In the present work only *S. stroma* was found and it was concluded that *S. obvelata* did not occur in *A. sylvaticus* on Raasay, Ulva or Scalpay. The infection with *S. stroma* on the islands was much heavier than that recorded for *S. obvelata* at Oxford.

Elton records that 85 per cent. of the specimens of *A. sylvaticus* examined at Oxford were infected with *Nematospiroides dubius* Baylis, 1926, and also records finding this species in specimens from the Isle of Lewis (1934). In the present work no examples of this parasite were found. The species is relatively large, up to 13 mm. in length, and would not have been overlooked if it had been present. Thus it may be

concluded that this species does not occur in these islands. The Isle of Lewis is farther from the mainland than are Raasay, Ulva and Scalpay, and it is difficult to understand why this species is absent from these islands yet common on Lewis. It is possible that the much greater volume of traffic between Lewis and the mainland has made it easier for parasitic infections to spread to Lewis than to these smaller islands which are less frequented.

Clethrionomys glareolus

The nematode fauna obtained from this host was similar in the three islands, but slight differences were observed which appear significant :—

(1) The infection with *C. muris-sylvatici* was extremely heavy on Raasay, 80 per cent. of the specimens being infected compared with only 15 to 20 per cent. on Ulva.

(2) The infection with *H. glareoli* was again heaviest on Raasay, though the difference between the islands was not as marked as in the case of *Capillaria*.

(3) The infection with *L. wolgaense* was heavy on Ulva, 7 out of 18 specimens being infected, but only 1 out of 41 was infected on Raasay and none on Scalpay. In the case of Scalpay, the absence of this species may be due to the small number of animals examined, but considering the much larger number examined from Raasay it must be concluded that this parasite is only common on Ulva.

(4) *Aspiculuris tetraptera* was recorded from Ulva only, where it occurred in 3 out of 13 specimens examined. The fact that 41 specimens were examined from Raasay without finding the parasite indicates that it is rare or absent from Raasay. Again, its absence from Scalpay may be due to the small number examined.

(5) The single infection of *Trichuris muris* appears to be an accidental infection, and of little importance.

These considerations indicate that significant differences occur between the faunas of Raasay and Ulva. The number of specimens from Scalpay was too small to allow any conclusions to be drawn.

Elton examined a large number of specimens of *C. glareolus* from Oxfordshire, and found only *C. muris-sylvatici*, *H. glareoli* and *A. tetraptera*. Of the three species reported as new records for this host. *T. muris* is extremely rare, and though absent from Elton's material, this species may be present generally in *C. glareolus* in small numbers. *T. retortaeformis*, however, was present in about 10 per cent. of the

specimens examined in the present work, and *L. wolgaense* in 15 per cent.

C. muris-sylvatici was reported by Elton from *C. glareolus* at Oxford, but was found in only one specimen. In the present work 80 per cent. of the specimens from Raasay harboured the parasite, thus this species is much more common in this host on the islands than in Oxfordshire. In this connection it is interesting to note that the *A. sylvaticus* examined in this survey also showed a much higher incidence of infection with *C. muris-sylvatici* than those examined at Oxford.

T. retortaeformis is common in rabbits and hares in England and Scotland, and so its occurrence in *C. glareolus* can be readily understood. It was not recorded from Oxfordshire, but is probably widespread as a parasite of *C. glareolus* wherever this latter species and rabbits live in close proximity.

L. wolgaense was quite common in the material from the islands but has not been reported elsewhere in Britain. Very little work has been done on the parasites of *C. glareolus*, however, and it is probable that it occurs in other areas. The species was not found by Elton, and it must be concluded that it is absent from *C. glareolus* in Oxfordshire.

In general the nematode faunas of *A. sylvaticus* and *C. glareolus* examined in this work show a greater tendency to overlap than was found at Oxford. This may be related to the more restricted area and subsequent restriction of the range of environment available on these islands.

Sorex araneus.

The specimens examined from this host show a similar trematode and nematode fauna from all three islands. The infections appear heaviest on Raasay, and one species, *L. trus*, is recorded only from Raasay, but this may be explained by the disproportionate numbers examined from each island. The parasite fauna of *S. araneus* was found to be completely different from that of the *A. sylvaticus* and *C. glareolus*, no overlapping occurring. The parasite fauna of *S. araneus* was much more varied, 36 per cent. being infected with trematodes, and 8 species of nematodes being found. The nematode fauna was of considerable interest, and included three new species all of the genus *Longistriata*.

Previous work on the trematode and nematode parasites of *S. araneus* has been very limited. Baylis (1928) recorded the occurrence of two trematodes, *Distoma migrans*, Duj., 1845 and *D. exasperatum*, Rud., 1819, and two nematodes, *Capillaria incrassata* (Diesing, 1851) and

Longistriata depressa. *C. incrassata* is found in the bladder, and is therefore outside the range of this survey. Morgan (1928) examined one specimen from St. Albans and recorded the occurrence of *P. winchesi*, and Baylis and King (1932) examined a specimen from Millport and described *Soboliphyme soricis*, but no other parasites were reported in either case. Two of the new species of *Longistriata* recorded in this paper are more common on the islands than *L. depressa*, and it may be that they are present in other areas also, but that they have so far escaped notice, possibly because of their small size and superficial resemblance to *L. depressa*.

Summary.

(1) A survey was made of the trematode and nematode parasites of *Apodemus sylvaticus*, *Clethrionomys glareolus* and *Sorex araneus* from the islands of Raasay, Ulva and Scalpay.

(2) The following species have been recorded :—

A. sylvaticus :

Trematodes—

Lyperosomum vitta
Brachylaemus recurvum

Nematodes—

Capillaria muris-sylvatici
*Heligmosomum glareoli**
Syphacia stroma

C. glareolus :

Nematodes—

Capillaria muris-sylvatici
*Trichuris muris**
*Trichostrongylus retortaeformis**
Longistriata wolgaense†*
Aspiculuris tetraptera

S. araneus :

Trematodes—

Brachylaemus fulvus†

Nematodes—

Parastrongyloides winchesi
Capillaria exigua
Longistriata depressa
Longistriata codrus†

Longistriata trus†
Longistriata didas†
Soboliphyme soricis
Porrocaecum (*spirale* ?) larva

* New host records.
† Species new to Britain.

(3) The female of the species *Longistriata wolgaense* is described for the first time.

(4) Three of the species recorded, *L. codrus*, *L. trus* and *L. didas* are new species and are described in this paper.

(5) The results of the survey are discussed and compared with records from other areas of Britain.

ACKNOWLEDGMENT.

The author wishes to acknowledge the helpful criticism and encouragement he has received from Dr. H. D. Crofton, under whose supervision the work was carried out.

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Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, pp. 169—180.

A Study of the Conditions Favouring the Survival *in vitro* of the Cattle Lungworm, *Dictyocaulus viviparus*

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In reviewing the literature on helminthology one finds that a great deal of attention has been given to the study of the physiology of parasites. Investigations of such a nature are valuable, in that they enable one to approach the problem of anthelmintic treatment in a scientific manner. Most of the early work that was carried out on lungworms in general was concerned either with their life histories or with their morphology, and little work has been done on their physiological characteristics. As a result all the drugs used so far in the hope of expelling these parasites from the lungs have been employed empirically. The use of treatments such as intra-tracheal medications or fumigations with elements such as sulphur or iodine are examples of these.

Parasitic nematodes in general are quite strongly resistant to adverse environmental conditions, and in the case of *Dictyocaulus viviparus*, the mature adult parasites can sometimes be observed alive in the lungs for the first 24 hours after the death of the host. The physiological reactions of the parasite are influenced by their local environment and will vary according to whether they have been removed from the lungs of a freshly dead animal or sometime subsequent to death. In the latter case they themselves will be moribund ; thus before they can be studied properly it is essential to evolve artificial media in which they can be kept alive in a normal healthy state. Experimentally it has only been possible to reproduce the complete life cycle *in vitro* with two nematodes. These two are both parasitic in insect hosts, i.e. *Neoaplectana glaseri* from the Japanese beetle (*Popillia japonica*) reared *in vitro* by Glaser (1931 and 1932) and the allied species of parasite *Neoaplectana chresima*, whose life cycle was also reproduced by Glaser, McCoy and Girth (1942).

Brand and Simpson (1944) kept *Eustrongylides ignotus* larvae alive *in vitro* for two and a half years. This is a tissue parasite in the fish (*Fundulus heteroclitus*) whose adult forms are to be found in the black-crowned night heron (*Nycticorax nycticorax hoactli*). *Camallanus americanus* was kept alive for about two months by Magath (1919), *Ascaris lumbricoides* for about 26 days by Hall (1917). These examples indicate that species parasitic in cold blooded animals seem to live longer in artificial media.

Purpose of the Study.

The work was undertaken to study the conditions favouring the survival *in vitro* of the adult stage of *Dictyocaulus viviparus* with special reference to its saline requirements. Fenwick (1939) investigated the larvae of *Ascaris suum* in very much the same way. The mature adult fifth stage of *Dictyocaulus viviparus* was the stage chosen with which to carry out this study for the reason that if there is any chance of developing an effective anthelmintic against this parasite, it is this stage against which it should be lethal. This is a very important feature apart from its academic significance. When considering the appropriate medium to be employed for the survival of these nematodes, it is desirable in the first instance to simulate as far as possible the chemical and physical properties of the host environment in which the parasite lives. A review of the work previously done on the effect of varying concentrations of inorganic salts on animal tissue by Lewis and Lewis (1911) and also a review of the work done on parasitic forms by Lapage (1935), Hoeppli, R., Feng, L. C., and Chu, J. (1938), Stoll (1940) and Fenwick (1939), suggested that firstly it was highly important to study the most suitable ionic concentrations of NaCl, KCl, $CaCl_2$ and $MgCl_2$ for the parasites.

Material and Methods.

The mature adult parasites of *Dictyocaulus viviparus* were collected from the lungs of cases of husk that were reported to the laboratory during the summer of 1951. The material was secured freshly from cattle that were killed either at the knackeries or the laboratory. The trachea and its branches were opened longitudinally with a pair of scissors and adult parasites were collected by means of two dissecting needles held parallel. They were then put into physiological saline at a room temperature of 20°C in wide, shallow Petri dishes. The parasites were cleaned of mucus exudate and washed several times in

the respective saline concentrations in which they were to be experimented with, again using sterile, wide shallow Petri dishes. When this technique was working properly the material could be prepared in about one hour. Parasites were first checked for their normal morphological appearance. They were selected according to their state of maturity and were observed to be moving vigorously in the physiological saline. Sterilized pots with screw-top caps containing the different concentrations of saline in which the worms were to be kept were used for culturing. Each pot contained 50ccs. of the required saline. Two sets of experiments of each concentration of salt were used, one at room temperature 20°C and one in the incubator. To each of these pots five adult parasites were introduced taking care to guard against contamination. The time was recorded and they were observed every 12 hours. These parasites were observed to feed by ingesting the lung exudate (Soliman, 1951), a fact that makes it almost impossible to sterilize them as they may harbour bacteria in their alimentary tracts. Thus it was thought advantageous to change the saline containing them every 24 hours, to minimize the bacterial contamination. When this saline was replaced it was of the same temperature and concentration as before in each case. The movements of the parasites differed a great deal in different salines but usually began with active movements and ended in death when they gave no response at all to stimuli, e.g. when touched with a glass rod.

Taking the movements as the fundamental basis for observation the stages through which the parasites passed up to the time of their death were classified as follows :

1. Active movements.
2. Slow movements.
3. Feeble or sluggish movements.
4. Moribund stage that preceded actual death where the only signs of life were very feeble movements at one or both ends (head or tail) without any body movement.
5. Actual death indicated by no response to stimuli.

The life span of the parasites differed in different salines. The rate of deterioration was uniform and rapid in all concentrations of saline but became appreciably slower after the parasites had reached the sluggish stage. Subsequently a marked variation was observed in the time taken to for them to die. Since this phase could not be considered

healthy or normal, dates concerning times of survival were only recorded up to the point when only feeble or sluggish movements were visible.

Each of these experiments was repeated four times whenever suitable material was available. The average time of survival of the parasites and the maximum survival time for individual ones for each series of experiments was worked out and is illustrated in diagrams (I–V). In this way a fairly accurate idea of the optimum ionic concentration of each saline was formed.

Results.

1. *Determination of optimal molecular concentration using sodium chloride.*

The first procedure taken was to find the most suitable osmotic pressure of saline. This would give an indication as to the optimum value for the total ionic concentration. As the parasites dealt with actually live in the lung exudate, sodium chloride was chosen as the most suitable salt to experiment with. Solutions were prepared in different percentages as follows :—

0.2, 0.4, 0.6, 0.7, 0.75, 0.8, 0.85, 0.9, 1.0, 1.1, and 1.2.

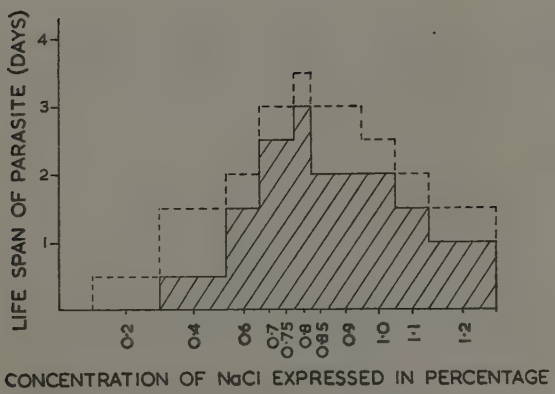
The parasites in the lowest concentrations, namely 0.2, became turgid with water, the solution being hypotonic. Their movements were at first free and vigorous but by the time the first observations were recorded, namely, at the twelfth hour after the beginning of the experiment, they were all dead and had ruptured cuticles. In the hypertonic saline solutions movements were slow, not of the lashing type as in the hypotonic and the average survival time was rather higher.

The most suitable ionic concentration in which the average survival time was at its height, was found to be between 0.8% and 0.85%. In these two concentrations the average life span was three days.

Graph I represents the results obtained for the average survival time for the four series of experiments made. The maximum survival for individual specimens is indicated by the dotted line for each particular concentration. In the other four series of experiments carried out at 37°C the maximum life span was shorter by a day than at 20°C for the optimum two concentrations previously mentioned (0.8% and 0.85%). In spite of changing the saline every 24 hours

bacterial contamination at 37°C was marked. Therefore experimentation at this temperature was not carried out subsequently.

On discovering the optimum ionic concentrations for the survival of these parasites to be between 0.8% and 0.85% NaCl the next step was to find out the optimal amounts of the following salts, $CaCl_2$,



Graph I. Effect of different osmotic pressures of NaCl solutions on adult mature *Dictyocaulus viviparus*.
Straight line = average results of four series of experiments.
Dotted line = maximum individual results obtained.

$MgCl_2$ and KCl when added separately to a solution of NaCl of 0.8% concentration. The lowest percentage of NaCl was chosen so that when any of the other salts were added the total ionic concentration of the final solution would remain within the optimal ionic concentration range, that is between 0.8% and 0.85%.

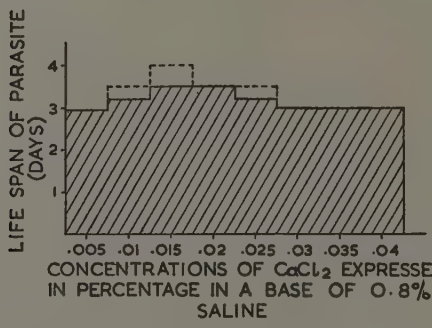
The Effect of Addition of Calcium Chloride in Small Amounts to 0.8% NaCl on the Survival Time of D. viviparus.

The addition of small amounts of $CaCl_2$ to sodium chloride solution 0.8% were studied in this experiment.

The solutions made up were :—

NaCl%		$CaCl_2$%
0.8	+	0.005
0.8	+	0.015
0.8	+	0.020
0.8	+	0.025
0.8	+	0.030
0.8	+	0.040
0.8	as	control.

Four series of experiments were repeated as previously. Graph No. 2 sums up the results obtained. The addition of $CaCl_2$ to the saline



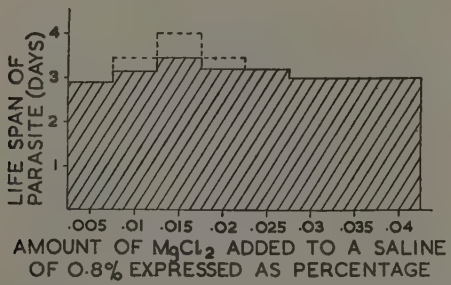
Graph II. Effect of addition of small amounts of $CaCl_2$ to a base solution containing 0.8% NaCl.
Straight line = average results of four series of experiments.
Dotted line = maximum individual results obtained.

resulted in a lengthening of the life span of the parasite up to a maximum of half a day in a saline containing 0.01–0.020% of this salt. Decrease in the concentration to 0.005 or increase to 0.025, although still beneficial in lengthening the average survival time over the simple

saline of 0.8% was less than the average when $CaCl_2$ was added in amounts of between 0.01% and 0.02%.

The maximum survival time for individuals was four days when $CaCl_2$ was added in 0.01%–0.015%. The optimal percentage of $CaCl_2$ was thus taken as 0.015% being the middle figure in the three concentrations 0.01%–0.015% and 0.020% and as it is in this concentration that the maximum life span for individual parasites was met with.

It is interesting to note that there was no toxic effect of this salt in the percentages tested as the average survival time remained at three days all through the experiment.



Graph III. Effect of addition of $MgCl_2$ in small amounts to 0.8% saline on the survival of *Dictyocaulus viviparus*.
Straight line = average survival time of parasite.
Dotted line = maximum individual survival time.

The Effect of Addition of Magnesium Chloride in Small Amounts to 0.8% Saline on the Survival Time of D. viviparus.

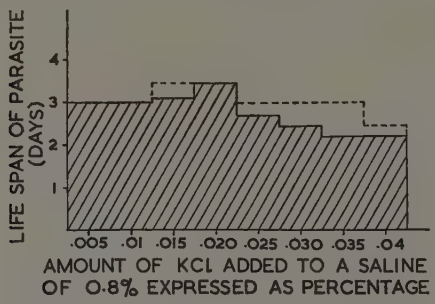
The previous experiment was repeated using $MgCl_2$ instead of $CaCl_2$. The solutions made up were :—

NaCl%		$MgCl_2$%
0.8	+	0.005
0.8	+	0.010
0.8	+	0.020
0.8	+	0.025
0.8	+	0.030
0.8	+	0.040
0.8	as	control.

Again four series of experiments were carried out. Graph No. III

sums up the results obtained. The addition of $MgCl_2$ exerted slight effect on the life span of the parasite in concentrations of 0.005 to 0.025%. Outside this range this salt had no effect. The highest average survival time was obtained with a percentage of 0.01. Thus this percentage was considered to be the optimum antagonistic ionic concentration of this salt.

The maximum survival time for individuals was also obtained with this concentration, namely 0.01% $MgCl_2$.



Graph IV. Effect of the addition of KCl in different percentages to saline of 0.8% on the survival of *Dictyocaulus viviparus*.
Straight line = average survival time of parasite.
Dotted line = maximum individual survival time.

The Effect of Addition of Potassium Chloride to 0.8% Saline on the Survival Time of D. viviparus.

The previous experiment was repeated using KCl instead of $MgCl_2$. The solutions made up were :—

NaCl%		KCl%
0.8	+	0.005
0.8	+	0.010
0.8	+	0.015
0.8	+	0.020
0.8	+	0.025
0.8	+	0.030
0.8	+	0.040
0.8	as	control.

Four series of experiments were carried out. Graph IV sums up the

results obtained. The addition of KCl exerted slight effect on the life span of the parasite in concentration of 0.015% for half a day. This salt when added in concentrations less than this had either very slight effect, as expressed in Graph IV in a concentration of 0.01% or exerted neither harmful nor beneficial effects of any sort. When added in concentrations higher than 0.015% the average survival time went beyond three days although the maximum survival for certain individuals was three days. Thus in concentrations above 0.015% a somewhat harmful effect is exerted as indicated by the lower average time.

Thus 0.015% KCl was considered the optimum antagonistic ionic concentration of this salt as it resulted in the highest average survival time when added to 0.8% solutions of sodium chloride.

The Effect of Addition of Optimal Ionic Concentrations of $CaCl_2$, $MgCl_2$ and KCl to 0.8% Sodium Chloride.

A saline containing 0.8% of sodium chloride plus the optimal percentage dilution of ionic concentrations found in the previous experiments of $CaCl_2$ and $MgCl_2$ and KCl was prepared. It was made up as follows:—

0.8% NaCl + 0.015% $CaCl_2$ + 0.010% $MgCl_2$ + 0.015% KCl

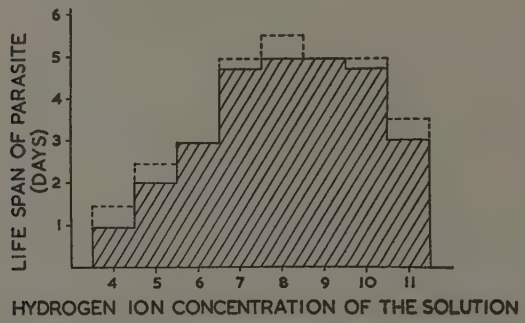
This experiment was repeated four times, the average life span of the parasite in each of the four trials being five days. The maximum survival time for individuals in the solution was five and a half days. The average survival time in this solution was two days more than in normal saline alone and one and a half days more than in any of the salines of 0.8% strength, with one of the three salts $CaCl_2$, MgCl and KCl, added in optimal ionic concentrations as found in the previous three experiments.

It is thus evident that although the addition of each of the salts mentioned to the simple saline solution lengthened but little the average life span of the parasite, yet when these three salts were added together to saline of 0.8% this resulted in an appreciable increase in the average survival time. It can thus be concluded that the addition of the three different salts exerted a beneficial effect through the antagonistic action of the various ions present in these solutions.

The Effect of Variations of the Hydrogen Ion Concentration on the Survival Time of D. viviparus.

Lapage (1935) tested the effect of different *p*H values on the third stage larvae of the Trichostrongylids. The media he used for culturing these larvae ranged from *p*H 3.6 to 9.5. He found that they were quite tolerant to variations about *p*H5.0, but not to any decrease of *p*H below 5.0.

Having developed a saline in which the adult mature *Dictyocaulus viviparus* could be kept alive in a more or less healthy condition it was thought advisable to find out the tolerance of the parasite to *p*H variation of the previously mentioned evoluted saline.



Graph V. Effect of variations of *p*H on the adult *Dictyocaulus viviparus*.
Straight line = average survival time of parasite.
Dotted line = maximum individual survival time.

A solution was prepared as follows :—

0.8% NaCl + 0.015% $CaCl_2$ + 0.010% $MgCl_2$ + 0.015% KCl

This was buffered to values ranging from 4 to 11 in round figures only. Again 50ccs. of each of these was put in a sterile pot and five mature normal adults were placed in it and left at room temperature, 20°C. The different solutions were changed every 24 hours so as to ensure stability of the *p*H as the by-products or secretions of the parasites might influence it. These pots were checked every 12 hours. Determination of the *p*H was conducted by means of capillator with a range of *p*H 4–11.

Four experiments were performed to ensure constancy of results. Graph No. V shows that adult *Dictyocaulus viviparus* tolerated a *p*H range of 7 to 10 without serious effects. The optimal *p*H was 8, at which the parasites lived for five days. In salines buffered to greater acidity than 7 the duration of life was shortened very distinctly. A range of *p*H 8 to 10 had only a slight adverse effect on the parasites but when the range shifted towards the acid side, that is, below *p*H 7, the deleterious effect was more rapid. Thus it seems that *p*H 8 is the most suitable hydrogen ion concentration for these parasites under the conditions of the experiment.

Conclusions.

1. The most suitable molecular concentration for the *in vitro* survival of mature *Dictyocaulus viviparus* lies between 0.8% and 0.85% sodium chloride.

2. The addition of $CaCl_2$ in small amounts to 0.8% NaCl exerted a beneficial effect by lengthening the survival of the parasites. The optimal amount to be added was found to be 0.015% $CaCl_2$.

3. The addition of small amounts of $MgCl_2$ to 0.8% NaCl exerted a slight effect in lengthening the survival time. The optimal amount was found to be 0.01% $MgCl_2$. The addition of this salt decreased the toxicity of the sodium ion to a small degree ; it had no deleterious effects.

4. The addition of small amounts of KCl to 0.8% NaCl exerted a slightly beneficial effect on the life span of the parasite in a concentration of 0.015% KCl. The addition in this percentage decreased the toxicity of the sodium ion but when added in higher concentrations than this it exerted a reverse effect.

5. The most suitable saline for the survival of *Dictyocaulus viviparus in vitro* under the conditions of the experiment was found to be :—

0.80% NaCl + 0.015% $CaCl_2$ + 1.010% $MgCl_2$ + 0.015% KCl

The life span of the parasites in this solution was five days.

6. The optimal *p*H of this medium was found to be 8.0.

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Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, pp. 181—184.

The Root-Knot Eelworm on Weeds and Cultivated Plants in the Gold Coast.

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Intensive cropping on a commercial scale as practised on fertile land in Europe is hitherto unknown in the Gold Coast apart from the growing of cocoa trees in a wide belt stretching from east to west across the central region of the country. In general, the method of farming is exceedingly primitive, being largely shifting cultivation, that is, the bush is partially cleared and the land is then cultivated by hand-hoes and cropped for a period of three or four years. Normally, no manure of any kind, either organic or inorganic, is applied and the land at the end of this period is allowed to revert to bush. New ground is brought into cultivation in its place, only to be allowed when virtually exhausted of readily available plant food to return to its natural, jungle condition.

Recently, attempts have been made, as in other territories in Africa, to develop by means of modern equipment a system of intensive cropping of the land with a view to meet requirements of the rapidly increased population and the urgent need for expanding the export trade. Pilot experiments under the auspices of the Agricultural Development Co-operation are being carried out and already one of them is virtually doomed to failure due to an unexpected presence of the root-knot nematode (*Meloidogyne* sp.) in the soil. In this case, several acres of fertile land at Abessey, some ten miles north of Accra, was cleared in early 1953 of all trees and bushes and planted with tomatoes, cabbages, swedes, carrots, lettuces, beans, melons, cucumbers, pepper and garden-egg plants. The principal crop, tomato (*Lycopersicum esculentum*), failed to produce any marketable fruit and the plants remained spindly and yellowish green in colour. The plants grew to a height of about three feet and then the foliage suddenly withered, became brown, shrivelled up and died. They were in this condition when examined by the writer in mid-May, 1953, and their roots displayed all the characteristic features associated with a serious attack of the root-knot eelworm. The result of the microscopic examinations made later in the laboratories at the University College of the

Gold Coast confirmed the presence of this nematode in enormous numbers and in all stages of development from egg to adult.

The tomato plants consisted of two varieties. Rutgers and Marglobe, both being wilt-resisting types. They had been grown from seed obtained from the United States of America and sown in a seed-bed on the land in which the seedlings were later transplanted. An examination of the roots of the other cultivated plants growing alongside the tomatoes revealed light to moderate infestations by the root-knot eelworm on :—

COMPOSITAE :	Lettuce (*Lactuca sativa*).
CRUCIFERAE :	Cabbage (*Brassica oleracea capitata*). Chinese cabbage or Wongbok (*Brassica pekinensis*). Swede (*Brassica rutabaga*).
CUCURBITACAE :	Cucumber (*Cucumis sativus*).
LEGUMINOSAE :	Runner or pole bean (*Phaseolus multiflorus*).
SOLANACEAE :	Garden-egg (*Solanum melongena*, var. *ovigerum*). Pepper (*Capsicum annuum*).
UMBELLIFERAE :	Carrot (*Dancus carota*).

All these crops had been sown much later than the tomatoes and the lighter degree of infestations noted on them may have been associated with this factor rather than with a lighter degree of resistance by them to attack by this nematode.

In the attempt to discover the source of the infestation, a careful search was made of the roots of weeds growing among the cultivated plants and in waste places in the vicinity of the parcel of land developed for intensive cropping. The presence of the root-knot eelworm was detected on dissection and microscopic examination of galls on the roots of the following weeds indigenous to the Gold Coast :—

CAPPARIDACEAE :	*Cleome ciliata*, a wild spinach, at least a species of plant not used for human consumption in the Gold Coast.
EUPHORBIACEAE :	*Acalypha ciliaris*, a common weed of cultivated land in forest regions.
FICOIDACEAE :	*Trianthema portulacastrum*, a common weed of waste places.

There can be no doubt that these three prevalent species of weeds act as reservoir hosts for the root-knot eelworm in the Gold Coast and that they constituted the source of the outbreak on the cultivated crops grown on the land made suitable in early 1953 for intensive cropping at Abessey, Accra. It was evident that the nematode had no difficulty in establishing itself on these economic plants grown for human consumption. For instance, it was found that tomato seedlings about four weeks old and only some three to four inches high growing in a seed-bed, on part of the ground most recently converted into a suitable state for arable cropping, contained an abundance of minute nodules, but quite obvious to the naked eye, on their roots. Appreciable numbers of the root-knot eelworm were present within these galls set up on the roots, and a high proportion of the female worms had already reached maturity and given rise to embryonated ova.

In order to improve the physical condition of the soil and simultaneously increase its organic content, a system of green manuring had been adopted on this land brought into cultivation for the intensive production of food for human consumption. The first crop grown after clearing the land of all bush and rough herbage and bringing it into a fit state for arable cropping consisted of a quick growing variety of cattle turnip (*Brassica rapa*). The seed was sown thickly with a view to obtain a good covering for smothering the weeds and at the same time provide an abundance of luxuriant growth of sappy, tender foliage for ploughing into the ground as green manure. A critical examination of the root system of such a crop ready for incorporation into the soil, on the most recently cleared land, revealed that the root-knot eelworm had set up small galls on the rootlets of about 10 per cent. of the plants. It was evident that the practice of growing this crop, though highly beneficial for improving the physical state and fertility of the land, had aggravated the eelworm problem as it had actually led to a considerable increase in the degree of infestation of the soil. Under Gold Coast conditions, a period of about 30 days is sufficient for the completion of the life-cycle of this nematode, from the larva to the egg-laying stage, in cruciferous plants, at least in turnips, swedes and cabbages.

Although the time needed in the Gold Coast for the completion of the life-cycle of the root-knot eelworm is approximately the same on these brassicae as on tomatoes, the extent of the root manifestations and sensitivity of the plant to attack is far greater in the case of the latter. It is realised that certain high yielding varieties of tomatoes may be more susceptible to serious injury than others, particularly the

less productive types. It is noteworthy in this connection that tomatoes of inferior quality have been grown in the Gold Coast on a very limited scale for, at least, half-a-century, and, it is claimed, without serious trouble from any disease. In view of the fact that the original tomato crop which proved a complete failure at Abessey due to severe infestation by the root-knot eelworm had been raised from imported seed, it was decided to test on the same land a local variety cultivated in the Gold Coast for a very long time. When the plants of this variety were examined in an advanced seedling stage, it was found that their roots were heavily infested and that the female worms had already laid masses of eggs into their gelations exudations. There was no evidence that these plants of the local variety showed more resistance to the parasite than those raised from seed of the two imported varieties Marglobe and Rutgers.

The search for root-knot eelworm was extended to the surrounding district and other localities. Its presence was detected in the roots of a cultivated variety of cassava, *Manihot utilissma* of the natural order Euphorbiaceae, growing within some 400 yards of the land cleared for intensive cropping and where the tomatoes and other crops had been found severely infected with the nematode. Cassava has been grown from time immemorial in the area and it constitutes the staple diet of the people over the greater part of the Gold Coast. The presence of the root-knot eelworm was also discerned in the roots of the aforementioned weeds, *Acalypha ciliaris*, *Cleone ciliata* and *Trianthema portulacastrum* at Pokoasi and Mayara, some three and seven miles, respectively, from Abessey where it was first noted. In neither case were the weeds growing among cultivated plants and there was no evidence that the land had been under cultivation for a long time, if ever.

Grateful acknowledgments are due to Mr. J. P. Mayhew, Department of Agriculture of the Gold Coast, for his helpful co-operation in connection with the field observations at Abessey.

Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, pp. 185—198.

*On *Fasciola indica* n.sp. with Some Observations on *F. hepatica* and *F. gigantica*.

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Indian flukes of the genus *Fasciola* have hitherto been studied only superficially. Bhalerao (1935) recorded the occurrence of both *Fasciola hepatica* and *F. gigantica* in certain parts of India, but his descriptions and illustrations leave much to be desired and these are the only ones available in the literature of the Indian species of *Fasciola*.

Having at hand quite a large number of specimens of these trematodes from India and other countries, the writer made a critical study of these inadequately described Indian forms. This study furnished some new morphological data concerning *F. hepatica* Linnaeus, 1758, and *F. gigantica* Cobbold, 1855, and resulted in the identification of the Indian flukes as a new species. Specimens from Rangoon, Singapore and Malaya were found to correspond in morphological details with the Indian form. This new fluke forms the main subject of this paper in which it is described and compared with other species.

Historical Review.

The history of " common liver fluke " (*F. hepatica*) is traceable from a period as early as 1526, when Cosimus observed an epizootic of distomatosis in sheep in Holland (quoted from Stiles, 1894). Linnaeus in 1746 proposed the genus *Fasciola* for it, and in the tenth edition of his *Systema Naturae* (1758) it appeared under this name.

Cobbold (1855) discovered another species in the liver of a giraffe which he named *Fasciola gigantica*. He differentiated it from *F. hepatica* on account of its greater size and elongation and the presence of more

*Part of a thesis approved by the University of London for the award of the Ph.D. degree.

numerous secondary intestinal branches. He also indicated that the posterior half of the body of *F. hepatica* was gradually narrowed presenting a more or less V-shaped outline, while in *F. gigantica* the narrowing only commenced at a very short distance from the tail end, which in some instances was blunt or even truncated. In certain cases the position of the " foramen caudale " was significantly marked by a notch at or near the centre of the termination of the tail.

Railliet (1895) described an elongated and slender form from the bile ducts of oxen slaughtered at Saint Louis in Senegal and distinguished it from the European liver fluke (*F. hepatica*) by its more elongated shape, lesser breadth (in the contracted state 26 to 38 mm. long and 6 to 8 mm. broad), short anterior cone, larger ventral sucker and larger eggs (143-151μ × 82-88μ, the average being 147 × 82μ). He named this form *F. hepatica* var. *angusta*.

The following year Looss (1896) recorded an analogous form from the bile ducts of cattle, buffaloes and sheep, slaughtered in Cairo and declared it to be a separate variety which he described as *F. hepatica* var. *aegyptiaca*. These flukes were 25 to 31 mm. in length and 6 to 7.5 mm. in breadth. The main distinctive features of this form, according to Looss, were the almost parallel lateral borders of the body ; the ovary and the testes were more branched ; the area occupied by the latter was much longer and the number of principal lateral branches and ramifications of the intestine were more numerous than in the European fluke (i.e. *F. hepatica*). The eggs measured 0.15 to 0.19 mm. in length but in breadth do not differ appreciably from those of *F. hepatica*, which are 0.13-0.14 × 0.075-0.09 mm. according to Leuckart.

Blanchard (1896) pointed out that Railliet's *F. hepatica* var. *angusta* and Looss's *F. hepatica* var. *aegyptiaca* were identical with Cobbold's *F. gigantica*. Looss (1902) returned to the subject and after examining a large number of specimens withdrew his species. Jackson (1921) reviewed the genus *Fasciola* with particular reference to *F. gigantica* and confirmed the identity of Railliet's and Looss's forms with Cobbold's species. He also gave an amplified description of *F. gigantica*.

Sinitsin (1933) described two new species of American liver flukes, *Fasciola californica* and *F. halli*, and differentiated them from *F. hepatica* by the distribution and form of the cuticular scales in the adults and by certain additional features in their life-histories. He further stated that *F. hepatica* was not found in America.

Material and Method.

The material on which the present study is based, consists of :

1. Several specimens of the new species described in this paper, collected by the writer from freshly slaughtered goats (*Capra hircus*) at Supaul and two buffaloes (*Bos bubalus*) at Purnea in Bihar (India) in April, 1952. They were fixed and preserved in formalin.

2. Specimens of English flukes (*F. hepatica*) collected by the writer in July, 1952, with the kind help of Dr. P. L. Le Roux from infected livers of cattle and sheep, obtained from abattoirs. They were fixed in formalin and stained fresh.

3. Specimens of the new species from cattle (*Bos indicus*) in Bihar (India), obtained from the collection of Bihar Veterinary College. They were preserved in formalin, but were not in a very good state of preservation.

4. Specimens of the new species collected by Prof. J. J. C. Buckley in Assam (India) in 1934 from cattle and buffaloes. These were preserved in separate tubes in formalin but were not in satisfactory state of preservation.

5. Specimens, identified in this study as *F. gigantica*, collected by Dr. P. L. Le Roux from cattle in Northern Rhodesia about 1940. They were preserved in formalin.

6. Specimens of Egyptian flukes, identified as *F. gigantica* in this study, obtained through the courtesy of the writer's colleague Mr. H. Gharib, M.R.C.V.S., of the Cairo Veterinary College. These were preserved in formalin.

7. Several mounted specimens of *F. hepatica* from Great Britain, *F. gigantica* from Africa and *F. indica* n. sp. from Malaya, available from the helminthological collection of the London School of Hygiene and Tropical Medicine.

The following specimens were available, through the courtesy of Mr. Prudhoe, from the British Museum (Natural History) :—

8. Five specimens labelled *F. gigantica*, from an ox in Accra, Gold Coast. They were preserved in 4% formalin.

9. One specimen labelled *F. gigantica* from cattle in Rangoon, preserved in spirit. This was found to correspond to the new species, *F. indica*, described in this paper.

10. One specimen labelled *F. gigantica* from an ox in Entebbe, Uganda, preserved in spirit.

11. One specimen labelled *F. hepatica* from a pig in Singapore, preserved in spirit. This also proved to be the new species described herein.

Most of the specimens were stained with acetic alum carmine and mounted in the usual manner. Those which were freshly collected took up the stain beautifully, but the old specimens did not stain well. Some specimens were stained with Ehrlich's haematoxylin also but the results were indifferent. With the carmine stain the digestive and the genital systems were brought up with different intensity and were stained dull red and bright purple respectively.

Fasciola hepatica Linnaeus, 1758 and *Fasciola gigantica* Cobbold, 1855.

Fasciola hepatica has been long known and carefully studied by a number of workers, but *F. gigantica* has received comparatively little attention. In differentiating the two species, much importance has been placed on the shape and size of the flukes. But Jackson (1921) in his revision of the description of *F. gigantica*, states that " the shape and size cannot count for a great deal in the delimitation of species in these forms owing to the distortion inevitably produced by maceration and fixation, unless coupled with distinct anatomical differences ". Nevertheless, his condensed description contains the following : " Body elongated and at least three times as long as broad ", and, " prominent shoulders always being absent and as a rule the sides of the body are roughly parallel and the posterior extremity is bluntly rounded or more rarely bluntly pointed ". In the writer's experience the shape and size are so variable, depending largely upon the fixation and stage of maturity of the fluke that much importance cannot be placed on these characters for specific purposes. Thus, with the object of augmenting the anatomical knowledge of these flukes, a comparative study of quite a large number of fresh specimens was undertaken and it revealed certain points of specific importance in respect of the system of gut-branching, the cuticular armature and the eggs.

Gut-branching. Previous authors have all recognised a rather simpler system of lateral and median diverticula of the gut in *F. hepatica* than in *F. gigantica* ; but none seem to have remarked that the lateral branches in the former tend to have a pronounced backward direction, in some examples almost parallel to the main stem (Fig. 1) whereas in

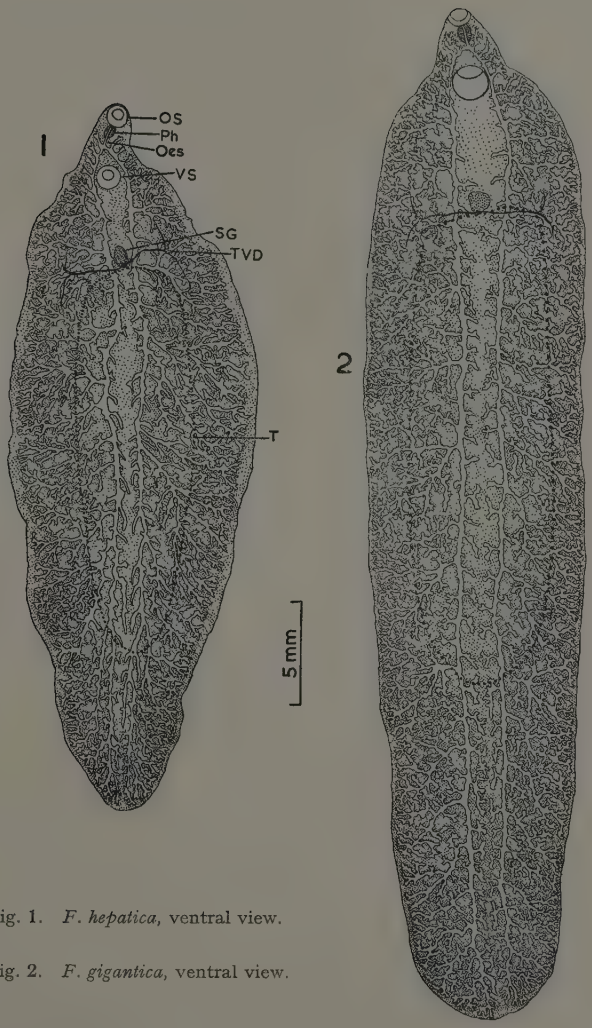


Fig. 1. *F. hepatica,* ventral view.

Fig. 2. *F. gigantica,* ventral view.

Abbreviations used in Fig. 1.

OS = oral sucker ; **Ph** = pharynx ; **Oes** = oesophagus ; **VS** = ventral sucker ; **SG** = shell gland ; **TVD** = transverse vitelline duct ; **T** = dotted line outlining testes.

F. gigantica they are more or less at right angles to the main stem (Fig. 2). Looss (1895) figures this character in his *F. hepatica* var. *aegyptiaca* and Faust (1930) in his illustration of *F. gigantica*, but these authors attach no specific value to it in their descriptions. With regard to *F. hepatica* also all text-figures show this character, but its possible significance has not been commented upon. In the present study the character was found to be remarkably constant in all the specimens of *F. hepatica* examined and although it is not so pronounced in *F. gigantica*, the difference can be easily appreciated visually.

Cuticular armature. Müller (1930) studied the cuticular armature of *F. hepatica* extensively and came to the conclusion that they are in reality " scales " and not spines, as referred to by previous authors. Sinitsin (1933) followed Müller in adopting this term and the writer agrees with these authors on this point. Müller's photographic illustrations of the scales of *F. hepatica* are valuable. It is also evident from his findings that the shape and size of these scales and their arrangement over the body in rows or otherwise are very variable from one part to the other and also from individual to individual, depending largely upon the fixation and stage of maturity of the specimen. In respect of the dimensions of these scales Müller states (*loc. cit.*) : " Their dimensions are the most accurately determinable and also exhibit a definite regularity of form and size ". His measurements however appear to have been taken from scales *in situ* on the body, over which they do not lie flat but are disposed obliquely like the piscine scales. To obtain a true picture of these, the writer thinks it is necessary that they should be removed from the cuticle. With this object in view some specimens of both *F. hepatica* and *F. gigantica*, fixed in 10% formol-saline and apparently in the same stage of maturity, were treated with 7½% caustic potash overnight to disintegrate the cuticle. Portions from the acetabular region were then teased out gently and examined. Scales which were freed in this way were found to be much bigger than they appeared to be while still attached to the body.

As stated above, the shape and size of these scales are so variable that standard measurements of them are impracticable. But a comparative study of them in *F. hepatica* and *F. gigantica* indicated that in the former they are more elongated and thinner, whereas in the latter they are stouter and broader at the root. These differences are seen in the camera lucida drawing of scales from the dorsal part of the acetabular region. (Figs. 5A, B and 6A, B). Their true significance can only be determined by a comparative study of scales from flukes

of the same age and in the same state of preservation.

Eggs. To study the intra-uterine eggs some specimens of both species were transferred to 70% glycerine-alcohol in the usual manner and left therein till the alcohol evaporated leaving behind the specimens in glycerine only. Eggs from the anterior region of the uterus of these specimens were dissected out separately in glycerine and mounted. Twenty eggs from each species were drawn under camera lucida and measured. The measurements were then analysed statistically to estimate the standard deviation and the standard error of the mean, which are tabulated in Table I. Eggs from the Northern Rhodesian specimens of *F. gigantica* were slightly smaller than those from the Egyptian and Gold Coast flukes, but were much larger than those of *F. hepatica*.

TABLE I.

Measurements (in microns) of Eggs of *F. hepatica* and *F. gigantica*.

	F. hepatica		*F. gigantica*	
	Length	Breadth	Length	Breadth
Range :	125–155	70–88	150–190	78–95
Mean : (average) ..	133.6	79.4	166.3	88.3
Standard deviation : ..	9.3	4.3	11.3	5.0
Standard error : (Mean)	2.1	1.0	2.5	1.1

The data in Table I confirm that the eggs of *F. gigantica* are much larger than those of *F. hepatica* and can be distinguished without much difficulty.

Fasciola indica n.sp.

The flukes collected by the writer from the buffaloes in Purnea and from several goats in Supaul area (Bihar) were examined and found to differ considerably from *F. hepatica* or *F. gigantica*. The specimens in the Assam collection of Prof. Buckley and the Rangoon, Malaya and Singapore specimens from cattle, buffaloes and pig respectively were also found to correspond to this apparently new form.

Occurrence. Bile ducts of cattle (*Bos indicus*) in Bihar, Assam, Rangoon and Malaya. Bile ducts of buffaloes (*Bos bubalus*) in Bihar and Assam. Bile ducts of goats (*Capra hircus*) in Bihar and bile ducts of pig (*Sus cristatus*) in Singapore.

External features. Fresh living specimens were usually greyish in colour. They are markedly contractile and the suckers, particularly

the acetabulum, are very strongly developed. The general contour of the worm varies greatly in preserved specimens, but presents an appearance intermediate between *F. hepatica* and *F. gigantica*. The two shoulders at the level of the acetabulum, so characteristic of *F. hepatica* and absent or indistinct in *F. gigantica*, are distinctly prominent in this worm. The anterior short conical portion is about 2.5 to 3 mm. long and is more massive than in the other forms. The maximum breadth of the body is attained at about the level of the transverse vitello-duct (in *F. hepatica* the maximum breadth is at about the level of the middle of the body) and then it gradually decreases in width to the posterior end, which is bluntly rounded. In *F. hepatica* the posterior narrowing is more marked presenting a more or less V-shaped outline and the caudal end is bluntly pointed; whereas in *F. gigantica* the sides of the body are, as a rule, roughly parallel and the posterior extremity is bluntly rounded.

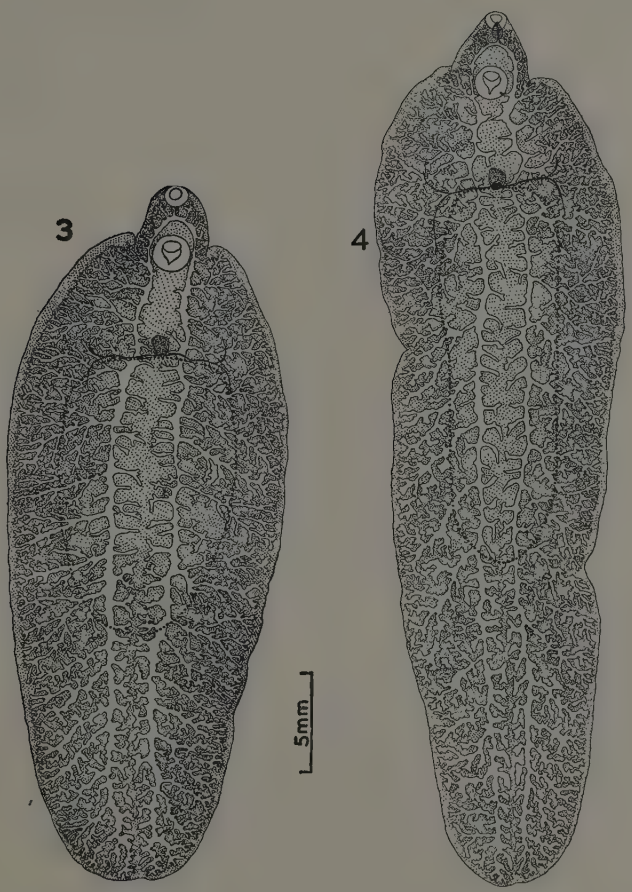
In the preserved specimens of *F. gigantica* the lateral margins of the body always show folds or wrinkles which are absent or insignificant in *F. hepatica* and the present species.

Size. Very variable since the worms reach sexual maturity long before they attain their maximum dimensions. Measurements of some apparently mature specimens are given below :—

1.	20 × 11 mm.	Bihar buffalo	Ratio of length to breadth	1.8 to 1.
2.	30 × 11.5 mm.	,, ,,	,, ,,	2.6 to 1.
3.	31.5 × 9.5 mm.	,, ,,	,, ,,	3.3 to 1.
4.	38 × 10.5 mm.	,, ,,	,, ,,	3.6 to 1.
5.	41 × 8 mm.	,, ,,	,, ,,	5.1 to 1.
6.	34 × 8 mm.	Bihar goat	,, ,,	4.2 to 1.
7.	29 × 9 mm.	Assam buffalo	,, ,,	3.2 to 1.
8.	30 × 10 mm.	Rangoon cattle	,, ,,	3.0 to 1.
9.	35 × 7 mm.	Malaya cattle	,, ,,	5.0 to 1.
10.	28 × 10 mm.	Singapore pig	,, ,,	2.8 to 1.

Thus, in average dimensions the worm shows affinity to *F. hepatica*, although some examples correspond to *F. gigantica*. As stated earlier, much importance cannot be placed on this character.

Cuticle. As in other species the cuticle of this worm also is covered with scales, which appear throughout the whole surface of the body with varying density. They differ from those in *F. hepatica* and *F. gigantica* in being broader, stouter and bluntly edged (Figs. 5c and 6c). Their variation in shape and size has already been indicated but it is



Figs. 3 and 4. *F. indica* n.sp., ventral view.

suggested that by a suitable comparative study the three species could be differentiated one from the other by this character.

Acetabulum. It is definitely larger than the oral sucker and its cavity is drawn backward into a blind triangular pouch. It measures about 1.7 mm. in diameter in preserved specimens and the thickness of

the wall in sections is about 0.5 mm. Its general structure agrees with that of other species of the genus.

Oral sucker. The oral sucker is well developed, placed at the anterior extremity slightly inclined ventrad. It is about 1 mm. in diameter and its muscular wall is about 0.85 mm. in thickness.

Between the oral sucker and the pharynx is found a "post-buccal ring", the depth and extent of which appears to depend largely upon the state of contraction of the specimen. But after examining some sagittal sections of the anterior end of the worm the writer concludes that ventrally it leads into a blind pouch, which, in structure, has great resemblance to that of the intestine. Thus, this confirms Mehlis' and Stiles' views in respect of *F. hepatica* and *F. magna* respectively.

Pharynx. The pharynx is well developed and opens into a very short œsophagus. The pharynx is about 1 mm. long and its muscular wall is about 0.4 mm. thick.

Intestine. (Figs. 3 and 4). The general dendritiform character of the alimentary system is typical of the genus, but in this species it takes a pattern intermediate between that characteristic of *F. hepatica* and *F. gigantica*. The principal lateral branches are greater in number (about 15) than in *F. hepatica* but not so many as in *F. gigantica*. Moreover, they take an intermediate course in their direction, being inclined latero-posteriorly at an angle of about 45 degrees with the main stem. The median branches also form a compromise between *F. hepatica* and *F. gigantica* in being more numerous and slightly ramose but not to the extent found in *F. gigantica*.

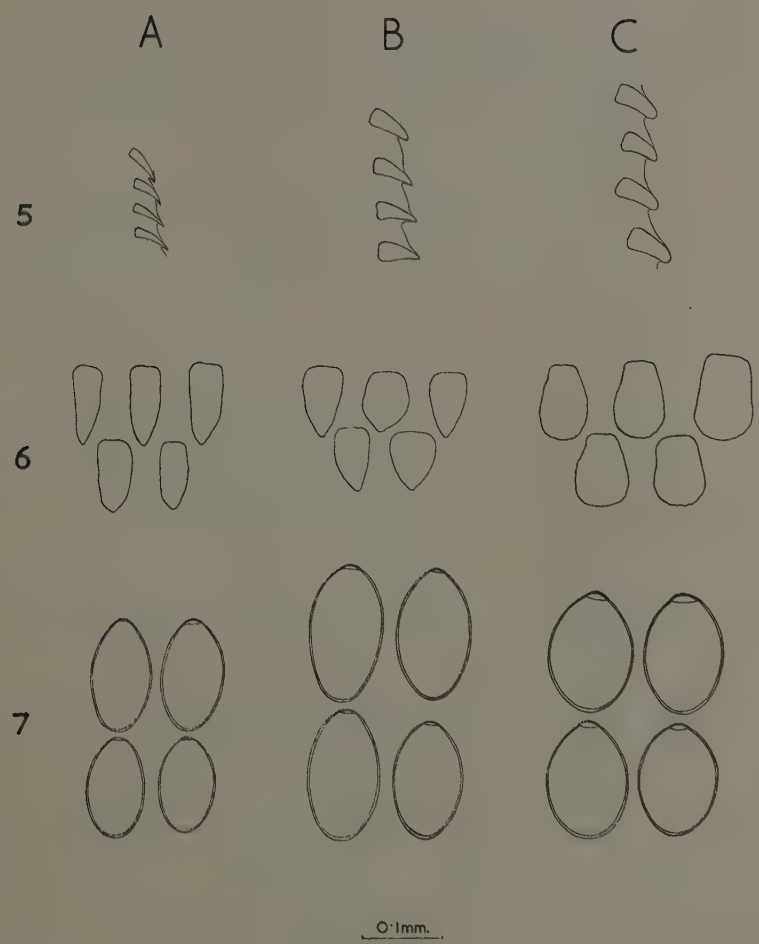
Genitalia. The genital organs resemble closely those of *F. hepatica* or *F. gigantica*, but the testicular area is much shorter in this form, occupying less than half the total length of the body. The testes are racemose and very well developed. The ovary, uterus, vitellaria, Mehlis gland, are the same as in related forms.

Eggs. The method of examining the intra-uterine eggs was as previously described and the measurements of 20 eggs were as follows:—

Range: 140–165μ (standard deviation 7.0) × 95–112μ (standard deviation 4.6).

Mean: 155.2μ (standard error ± 1.6) × 102.4μ (standard error ± 1.0).

Thus, these eggs are much broader than those of either *F. hepatica*



A = *F. hepatica*, B = *F. gigantica*, C = *F. indica*.

Fig. 5. Cuticular scales, lateral view. Fig. 6. Cuticular scales, dorsal view.

Fig. 7. Uterine eggs from anterior part of uterus.

TABLE II.

	F. hepatica	*F. gigantica*	*F. indica* n.sp.
General shape :	Prominent shoulders present. Maximum breadth of the body mid-way, which narrows posteriorly to a blunt point presenting a V-shaped outline.	Shoulders indistinct or absent. Sides of the body roughly parallel. Posterior end rounded.	Shoulders prominent. Anterior cone short and more massive. Maximum breadth of the body at the level of acetabulum. Decreases in width gradually to posterior extremity, which is rounded.
Length/breadth :	Roughly twice as long as broad.	At least three times as long as broad.	Intermediate between *F. hepatica and F. gigantica.*
Cuticular scales :	Fine, elongated and sharply edged.	Thicker, stouter and broader at the base.	Much more developed like true scales. Bluntly edged.
Acetabulum :	Almost equal to oral sucker or slightly larger than it.	Larger than oral sucker (about $1\frac{1}{2}$ times).	Larger than oral sucker (about $1\frac{1}{2}$ times).
Oesophagus and pharynx :	Oesophagus equal to or slightly shorter than pharynx.	Oesophagus shorter than pharynx.	Oesophagus much shorter than pharynx.
Intestine :	Lateral diverticula tend to incline posteriorly. Median branches fewer and simple.	Both lateral and median branches more numerous and racemose. The former directed more or less outwardly to the lateral margins.	Exhibits intermediate pattern in number, direction and branching.
Testes :	Profusely branched. Occupy about two-thirds of total body-length.	Racemose and more developed, occupying about half the total body-length.	Like *F. gigantica* but occupy less than half the total body-length.
Eggs :	125–155μ (SD 9.3) × 70–88μ (SD 4.3).	150–190μ (SD 11.3) × 78–95μ (SD 5.0).	140–165μ (SD 7.0) × 95–112μ (SD 4.6).
Distribution :	Europe.	Africa.	Oriental countries.

or *F. gigantica* and the difference is so significant that it can be appreciated even visually (Fig. 7c). The disparity between the breadth of these eggs and those of *F. gigantica* is very definite and the species can be identified by the breadth criterion alone. The operculum also is very prominent in this species.

Conclusion.

It is apparent from the above account that the species under discussion cannot be identified with any of the known species of *Fasciola* and that it shows closest affinities with *F. hepatica* and *F. gigantica*. The morphology of these three species is contrasted in Table II.

Summary.

1. *Fasciola indica* n. sp. from goats, cattle and buffaloes in Bihar is described and differentiated from *F. hepatica* and *F. gigantica*.

2. Specimens of *Fasciola* from Assam (cattle and buffaloes), Rangoon (cattle), Singapore (pig) were examined and identified as *F. indica*.

3. *F. hepatica* is further differentiated from *F. gigantica* on the basis of the cuticular armature and the disposition of the intestinal branches.

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Journal of Helminthology, Vol. XXVII, Nos. 3/4, 1953, *pp.* 199—205.

*The Parasitological and Pathological Significance of Arrested Development in Nematodes

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There seems to be no end to the variety and the complexity of host-parasite relationships concerning parasitism by nematode worms. Up to the present time comparatively little notice has been taken of the isolated observations of arrested development during parasitic life and no effort has been made to view them as a whole. It now appears that the phenomenon is of common occurrence throughout this group of parasites and is of interest to pathology as well as to helminthology.

It is an essential part of the ecological adaptation of almost all parasitic nematodes that they should be able to survive for a protracted period outside the final host, either as a larva enclosed in an egg shell, as a free living larva, or as a larva which is encysted within an intermediate host. Perhaps it is not surprising, therefore, to find that some infective larvae which have succeeded in gaining access to the final host should show a tendency to become dormant once again if any reaction of the host operates against their development. A number of isolated examples of this phenomenon have already been recorded, first among which should be mentioned Fulleborn's observation, reported in 1921, of the larvae of *Toxocara canis* remaining undeveloped for several weeks and probably for months in the liver of an unsuitable host, or for many days in the liver of the foetus until parturition takes place, after which they complete their migration and grow to maturity in the bowel of the newly-born puppy. Next might be mentioned the observations of Scott (1928) who found that third stage larvae of *Ancylostoma caninum* remained, without further development, for several weeks in the intestines of rats, dogs and cats before being eliminated. These larvae were found to be capable of development if transferred to a susceptible

*Received for publication 17th July, 1952.

host. This peculiar relationship of host and parasite had not to his knowledge been previously observed but he considered it probable that the use of the Baermann apparatus would show the presence of undeveloped worms in infections with other species. Gordon (1949) stated that he had found that the larvae of *Oesophagostomum columbianum* remained dormant in the mucous membrane of sheep for periods up to one year after the time of infection. An interesting example is also afforded by the larvae of *Physocephalus sexalatus* and of *Spirocerca sanguinolenta*. When an intermediate host beetle which contains these larvae is eaten by a vertebrate which belongs to an unsuitable host species the larvae re-encyst in the body cavity, in the mesentery or in the connective tissue surrounding the oesophagus—an observation that was first recorded by Seurat in 1916. This re-encystment may be repeated should the transport host be eaten by yet another unsuitable animal. *Syngamus trachea* provides an interesting variation, that of a larva which is already infective but is capable of encysting in the tissues of quite a number of invertebrates including various species of molluscs, earthworms and arthropods. These larvae are not particularly resistant to external conditions but as found by Taylor (1938) are capable of surviving for years in the invertebrate host. Conceivably this larva would be capable of re-encysting in predaceous invertebrates as for example in the body of slugs of the genus *Testacella* which feed on earthworms. Even *Trichinella spiralis*, the outstanding nonconformist of the nematodes, does at least fall in line with the rule for protracted dormancy in the larval state.

In 1949 Kotlan drew attention to the frequent occurrence of what he termed the *histotropic phase* in the development of nematode larvae in the vertebrate host and discussed some differences between various species during that phase in that some remain in the tissue only during their third larval stage and others remain there during the fourth stage as well. He also observed some " irregularities " in that sometimes larvae remain in the tissues for a much longer time than normal ; he had observed the larvae of *Hyostrongylus rubidus* to remain some months in the stomach wall. Similarly one of us (J.F.M.) has found the third stage larvae of *Trichostrongylus retortaeformis* when present in resistant rabbits, to remain undeveloped for as long as 98 days after the infective dose had been given to the rabbit. It was also observed that at varying intervals of time a few of these larvae resumed their development and grew to maturity. A still more interesting example is provided by T. E. Gibson, also of this laboratory, who has reached the conclusion

that fourth stage larvae and immature adults of the several species of *Trichonema* may remain dormant in the mucous membrane of the colon of the horse for months, and even for years. They appear to wait in this site of their encystment until such time as there is " living room " for them in the lumen of the bowel. If, for instance, an effective anthelmintic is administered to the horse and the adult *Trichonema* spp. are eliminated, then a comparable number of larvae re-enter the lumen of the bowel to take the place of the previous inhabitants. A report of these observations is now in the hands of the publisher.

Yet another example of this dormancy is provided by *Strongylus vulgaris*. Although little is known with certainty it appears that immature adults at an advanced stage of development are able to remain in the walls of the anterior mesenteric artery for long periods before finding their way to the intestine to complete their development.

The most recent addition to this list of observations of arrested development concerns lungworms of the genus *Dictyocaulus* and has been made at this laboratory during the course of an investigation of parasitic bronchitis among adult cattle. On account of the difficulty of working with *D. viviparus*, infestations of which tend to be quickly thrown off by our experimental calves, collateral observations were being made on *D. filaria* in sheep. This infestation is much easier to handle and it was while working with this parasite that we observed minute immature worms in the lungs up to 100 days after the administration of the infective larvae. Subsequent examinations of the lungs of adult cattle from outbreaks of acute parasitic bronchitis revealed the presence of similar microscopical worms (still in the earliest fifth stage of development), although the cattle had been away from the source of infection for some weeks. Experimental infections of cattle have since confirmed that the development of these worms may be arrested at what has proved to be the beginning of the fifth stage, and that they may remain in that condition for several months.

Work on the migrations of the Ascarididae by Sprent (1952) published since this article was prepared has provided further examples of the suspension of development. It appears that when embryonated ova of five species are fed to mice a somatic type of migration takes place, the larvae becoming more or less permanently encapsulated in various tissues.

Parasitological Significance of Arrested Development.

In all of these varied examples the worms may be regarded as waiting for suitable conditions in order to proceed with their development. The dormancy of eggs containing infective larvae, or of free-living larvae that have reached the infective stage is a relatively simple relationship of organism to unsuitable environment simulating the dormancy of seeds or of protozoan spores : that of larvae which have already taken up parasitic life in an intermediate host appears to imply a more complicated adjustment. The example seen in the two spirurid worms is still more complex as the larvae must actively leave the intestine of an unsuitable vertebrate host to re-encyst in the body cavity and so avoid possible destruction in the intestinal tract, or voidance into the outside world where chances of reaching the definitive host would be greatly reduced. This special adjustment provides them with a second chance and even a third or fourth chance of reaching the only environment in which maturity may be achieved.

Inhibited development such as has been observed in *Ancylostoma caninum, Oesophagostomum columbianum, Trichonema spp., Trichostrongylus retortaeformis* and the two species of *Dictyocaulus* fulfils the same function although it appears to imply a still more complicated adjustment. These larvae may be regarded as capable of maintaining themselves in a state of suspended development for several weeks until such time as the immune state of the host will permit of their further development. This is clearly indicated by Gibson's conclusions on *Trichonema spp.*, and by Scott's observations on *Ancylostoma*. J. F. M. has observed a tendency for dormant *T. retortaeformis* to develop to maturity a few at a time, and we have some evidence to show that the same kind of thing occurs in infections of the resistant host by *Dictyocaulus*. It seems clear that this faculty for dormancy in larval development is turned to advantage even in parasitic life where a temporary depression in the resistance of the host may be a not infrequent occurrence and provides a chance of growth and reproduction that would be denied to a parasite that had not acquired the mechanism for tiding over and time of unsuitable condition.

Pathogenicity of Worms Inhibited in their Development.

In most of the examples that have been given the presence of these dormant larvae is supported by the host with an amazing degree of tolerance. The absence of any obvious reaction in the tissues of invertebrate intermediaries appears to be the general rule and the

tissues of vertebrates frequently show no response. The larvae of the two spirurid worms above-mentioned cause no clinical disturbance and it would seem that *Trichinella* and *Trichonema* must be present in enormous numbers to bring about any pathological effect apart from local reaction round each individual larva. The same applies to *Toxocara canis* although Schillinger and Cram (1922) in an experimental infection *in utero* found 8 of the 12 puppies to be dead at birth. Inhibited development of larvae in the lumen of the stomach or intestine appears to have no pathological significance at all. The instance of *Strongylus vulgaris* is interesting in that although there are marked pathological changes in the surrounding tissues, very frequently involving the formation of a large aneurism, the health of the host is often unaffected.

In two of the examples, however, *O. columbianum* and *D. viviparus*, these arrested larvae can give rise to acute disease. In the case of *O. columbianum* the pathological reaction is associated with an acquired resistance in the host. The migration of the third stage larvae into the mucous membrane and back again into the lumen of the intestine proceeds without pathological incident in a previously uninfected host, but after the specific resistance has been acquired a pronounced reaction to the invading larvae is produced in the tissues and thick walled cysts result. In this relationship, therefore, we have an example of the curious position in which a certain aspect of a parasitic disease occurs only in the resistant host and not in the susceptible one. There appears to be a localized irritability of the tissues, which, once sensitized, respond to the penetration of the larvae of *O. columbianum* although for some strange reason they fail to do so to the larvae of *O. venulosum* which carry out the same kind of migration into the tissues and out. It has been suggested by the late W. E. Swales—in a private communication—that *O. columbianum* in the sheep is another example of a parasite in its wrong host and that it was originally a parasite of some species of deer. It will be seen that here the disease and the inhibition of development are both associated with the same cause, the formation of nodules.

With *D. viviparus*, however, although this direct relationship between the inhibition and the production of disease symptoms is absent, worms retarded in their development may yet give rise to disease. Our recent enquiries at Weybridge into the origin of the acute bronchitic symptoms in cattle, simulating those of parasitic bronchitis has shown that they are often accompanied by only a light

infestation of *Dictyocaulus viviparus* and that in some instances the adult worms cannot be found. Investigation by means of the Baermann technique, however, has revealed that microscopical worms are present in these instances, some in the fourth larval stage but mostly in the earliest period of the fifth stage of development. The actual numbers that we have recovered have not been very great, ranging only up to some 450, but because of the imperfection of the technique, the large size of the bovine lung and the relative inactivity of the larvae (differing greatly in that respect from the larvae of *Ascaris* which can be so plentifully recovered by this technique) it is thought that the microscopic *Dictyocaulus* must be very much more numerous than our technique shows. In experimental infestations subsequently carried out we have found these immature worms to remain in the lung up to the hundredth day after the administration of infective larvae to sheep, and up to the one hundred and sixtieth day in experimentally infected cattle.

From these observations we conclude that, as in the instance of the larvae of *Oesophagostomum columbianum* the pathogenicity of larval *D. viviparus* depends upon their invasion of sensitized tissue. At the first contact with infection cattle may show only slight symptoms or may gradually pass through the ordinary parasitic bronchitis syndrome, depending upon the numbers of infective larvae that they have picked up. If, however, the cattle have previously been infected, as is usually the case with adult cattle the sensitized lung tissue reacts violently to the second invasion of larvae, the lung becomes either oedematous or, more frequently emphysematous, sometimes to a very marked degree, and the animal shows symptoms of acute distress.

Perhaps, as in the instance of *O. columbianum* in sheep, this is another example of a parasite in its wrong host, *D. viviparus* may originally have been a parasite of deer. This suggestion receives some support from our experience of the difficulty in maintaining an infection in experimental cattle, which are not easy to infect while on a full diet and throw off the parasite very readily even when on a poor diet. This difficulty is not experienced with *D. filaria* in sheep and it seems not unlikely that some advantage might be gained by using deer as " culture hosts " for the continuation of our experiments.

Summary.

In summarising, therefore, we conclude that a tendency to become dormant during the larval stage, which is so characteristic a feature of

the free-living larvae of parasitic nematodes and is an essential requirement in their use of intermediate hosts is not an uncommon occurrence during their life in the final host. Its purpose in each instance is essentially the same, i.e. to carry it through a period in which the environment is unsuited to development ; on the ground, for instance, where it waits for a suitable host, as a parasite of an intermediate host, where it waits for a suitable final host, or in a resistant final host where inhibited development serves the parasite in enabling it to wait until some depression of the host's state of resistance allows it to grow to maturity.

In some instances these arrested larvae are the cause of pathological conditions in the host.

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Index to Volume XXVII

Index of Authors

New Names in Volume XXVII